Inequivalence of Statistical Ensembles

in

Single Molecule Measurements

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Abstract

We study the role of fluctuations in single molecule experimental measurements of force-extension $(f - \zeta)$ curves. We use the Worm Like Chain (WLC) model to bring out the connection between the Helmholtz ensemble characterized by the Free Energy $(F(\zeta))$ and the Gibbs ensemble characterized by the Free Energy $(G(f))$. We consider the rigid rod limit of the WLC model as an instructive special case to bring out the issue of ensemble inequivalence. We point out the need for taking into account the free energy of transition when one goes from one ensemble to another. We also comment on the “phase transition” noticed in an isometric setup for semiflexible polymers and propose a realization of its thermodynamic limit. We present general arguments which rule out non-monotonic force-extension curves in some ensembles and note that these do not apply to the isometric ensemble.

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**Introduction:**

In the past, experiments on polymers were confined to studying their bulk properties, which involved probing large numbers of molecules [1]. The results of these experiments could be analyzed by using the traditional tools of thermodynamics. In recent years, however, researchers have been successful in micromanipulating *single* biological molecules such as DNA, proteins and RNA to probe their elastic properties [2]. Such studies serve a twofold role. On one hand, they shed light on mechanical properties of semiflexible polymers, which are of clear biological importance in processes such as gene regulation and transcription [3–5]. On the other hand, they provide physicists with a concrete testing ground for understanding some of the fundamental ideas of statistical mechanics. In statistical mechanics, an isometric setup would be described by the Helmholtz free energy, whereas an isotensational setup would be described by the Gibbs free energy [6]. In the thermodynamic limit these two descriptions agree, but semiflexible polymers (those with contour lengths comparable to their persistence lengths) are *not* at the thermodynamic limit. Experimentally, both isometric and isotensational ensembles are realizable. Typically the polymer molecule is suspended (in a suitable medium) between a translation stage and a force sensor. The force sensor could be realized by using an Atomic Force Microscope (AFM) cantilever or by optical or magnetic forces. As noted by Kreuzer and Payne [7], an isometric setup can be realized using a stiff trap and an isotensational setup by using a soft trap. In a more sophisticated version, an electronic feedback circuit is used to control the force (or the extension) and one measures the fluctuations in the extension (or the force) [8]. Here we will focus on the role of fluctuations in single molecule experiments. In order to correctly interpret such experiments one needs to understand the effect of fluctuations on the measured quantities. For instance, it turns out, that an experiment in which the ends of a polymer molecule are fixed (isometric) and the force fluctuates yields a different result from one in which the force between the ends is held fixed (isotensational) and the end-to-end distance fluctuates [8,9]. This difference can be traced to large fluctuations about the mean value of the force or the extension, depending on the experimental setup. These fluctuations vanish only in the
thermodynamic limit of very long polymers.

Here we use the Worm Like Chain (WLC) model [10–12] to study the inequivalence of ensembles due to finite size effects. The WLC model has been very successful in achieving quantitative agreement with experimentally measured force-extension curves [2,13]. The paper is organized as follows. In Sec. II we discuss the Helmholtz and Gibbs ensembles. In Sec. III we consider the rigid rod limit which forcefully brings out the main issues dealt with in this paper. In Sec. IV we draw attention to the importance of taking into account the free energy of transition in going from one ensemble to another. In Sec. V we discuss the thermodynamic limit of a “phase transition” recently seen in semiflexible polymers. Finally, we end the paper with a discussion in Sec. VI.

II Helmholtz and Gibbs Ensembles:

Consider an idealized experiment in which one end of a molecule is held fixed at \((x_0, y_0, 0)\) and the other end is attached to a dielectric bead confined to a harmonic optical trap described by the potential

\[
V(x, y, z) = A \left[ \frac{(x-x_0)^2 + (y-y_0)^2}{2} \right] + C \left( \frac{z-z_0}{2} \right)^2.
\]

with \((x_0, y_0, z_0)\) defining the center of the trap. Consider \(A\) to be small so that the bead is free to move in the plane \(z = z_0\). For a polymer of contour length \(L\) and persistence length \(L_p\) it is convenient to introduce the following dimensionless variables: \(\zeta = \frac{x}{L}, \zeta_0 = \frac{x_0}{L}, \beta = \frac{L}{L_p}\) and \(f = \frac{FL_p}{k_B T}\) where \(F\) is an applied stretching force and \(k_B T\) is the thermal energy at a temperature \(T\). Consider \(P(\zeta) d\zeta\), the number of configurations (counted with Boltzmann weight) [11] for a polymer of length \(L\) starting from the origin and ending anywhere on the \(x - y\) plane in an interval \(d\zeta\) of \(\zeta\). The free energy defined by \(\mathcal{F}(\zeta) = -\frac{1}{\beta} \ln P(\zeta)\) is the Helmholtz free energy. The partition function [14] for the combined system consisting of the polymer molecule and the trap is given by:

\[
Z(\zeta_0, \beta) = \sqrt{\frac{C}{2\pi}} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} d\zeta e^{-\beta \mathcal{F}(\zeta)} e^{-\tilde{C} \left( \frac{(\zeta - \zeta_0)^2}{2} \right)^2}
\]

where \(\tilde{C} = CL^2\). By tuning the longitudinal stiffness \(\tilde{C}\) we can realize the two limiting cases.
**Helmholtz:** In the limit of a stiff trap ($\tilde{C} \to \infty$), the Gaussian factor pertaining to the trap approaches a delta function and one gets

$$Z(\zeta, \beta) = e^{-\beta F(\zeta)}$$  \hspace{1cm} (3)

Here we have switched notation to write $\zeta$ in place of $\zeta_0$. Thus a stiff trap realizes the Helmholtz ensemble by constraining fluctuations in the $\zeta$ coordinate. To extend the molecule from $\zeta$ to $\zeta + d\zeta$ one needs to apply a force $\langle f \rangle = \frac{\partial F}{\partial \zeta}$ in order to compensate for the change of entropy. Plotting $\langle f \rangle$ versus $\zeta$ we find the $(\langle f \rangle, \zeta)$ force-extension relation.

**Gibbs:** In the opposite limit of a soft trap ($\tilde{C} \to 0$ and $\zeta_0 \to \infty$ such that $\tilde{C}\zeta_0 = \beta f$ remains finite), one gets [14]

$$\tilde{Z}(f, \beta) = \int_{-\infty}^{+\infty} d\zeta e^{-\beta F(\zeta)} e^{\beta f \zeta}$$  \hspace{1cm} (4)

Thus a soft trap permits fluctuations in the $\zeta$ coordinate but constrains the force fluctuations [7] and thus realizes the Gibbs ensemble. $\tilde{Z}(f)$ is the generating function for the $\zeta$ distribution. Defining the Gibbs free energy $G(f) = -\beta \ln \tilde{Z}(f)$ we can work out the mean extension $\langle \zeta \rangle = -\frac{\partial G}{\partial f}$ and the $(\langle \zeta \rangle, f)$ force-extension relation.

Notice that $\tilde{Z}(f)$ is the Laplace transform of $Z(\zeta)$. In the thermodynamic limit of long polymers ($\beta \to \infty$) the Laplace transform integral Eq.(4) is dominated by the saddle point value and therefore $F(\zeta)$ and $G(f)$ are related by a Legendre transform:

$$F(\zeta) = G(f) + f\zeta.$$  \hspace{1cm} (5)

For finite $\beta$ i.e. for a polymer of finite extent, the saddle point approximation no longer holds true and fluctuations about the saddle point value of the free energy become important. Thus one finds that $F(\zeta)$ and $G(f)$ are not Legendre transforms of each other. We notice that this difference between the Legendre transform [Eq. (5)] and the Laplace transform [Eq. (4)] is the mathematical origin of the finite size fluctuation effects described in this paper. These fluctuations are of thermal origin and can ultimately be traced to collisions of the polymer molecule with the molecules of the suspending medium. In this section we
have recovered the results of [7]. We have also gone beyond [7] and traced the origin of the
difference between the two ensembles to the difference between the Legendre and Laplace
transforms.

**III Rigid Rod Limit: An Instructive Extreme Case**

We noticed in the last section that because of fluctuation effects the Helmholtz and Gibbs
free energies are not related by a Legendre Transform. An important consequence of this is
that the \((F, <z>)\) relation is different from the \((<F>, z)\) relation. In other words, the
force-extension curves plotted in the two ensembles are *distinct* due to finite size fluctuation
effects. Fluctuations dominate at finite \(\beta\) and disappear in the thermodynamic limit \((\beta \to \infty)\) of flexible polymers. We bring out the ensemble dependence of the force-extension
relations explicitly and most dramatically in the limiting case of a very stiff polymer \((\beta \to 0)\).

In this extremely rigid limit [15], the end of the polymer is uniformly distributed over the
sphere of directions. In the Helmholtz ensemble we thus have
\[
P(z)dz = \frac{dz}{2L} \quad \text{for } -L < z < L
\]
and \(P(z) = 0\) otherwise. Since the free energy is constant in the range \(-L < z < L\) and
diverges otherwise, we find that the average force \(<F>\) vanishes for \(|z| < L\) and diverges
for \(|z| = L\) (See Fig. 1a). In the Gibbs ensemble we find by standard manipulations that

\[
<F> = [L \coth FL \frac{1}{F}] \quad (6)
\]

which differs from the \(<F> - z\) relation determined above in the Helmholtz ensemble (See
Fig. 1b). The theoretical analysis of the ensemble dependence of the force-extension relation
based on the rigid rod limit is a new result of this paper.

Thus an experimenter making force-extension measurements on, for instance, Actin fila-
ments [16], would find that a measurement in which the force is controlled and the end-to-end
distance is measured leads to a different force-extension curve from a measurement in which
the end-to-end separation is controlled and the force is measured. A theorist interpreting
the curves also needs to keep in mind whether the curves are obtained in a constant-force
setup or a constant-extension setup since a proper interpretation of the curves requires a
knowledge of the ensemble used in the measurement.
IV Inequivalence Of Ensembles and the Free Energy Of Transition

In an isotensional setup one controls the force and one measures the mean extension and plots it as a function of force. In an isometric setup the roles of extension and force are interchanged. In both setups zero force corresponds to zero extension ($\zeta = 0$) and a large force corresponds to maximal extension (i.e. $\zeta = 1$). Imagine going from zero force to a large force via the isotensional setup and returning from maximal extension to zero extension via the isometric setup. Since the “equation of state” depends on the chosen ensemble, in general there will be two distinct curves describing the extension in one ensemble followed by contraction in the other [17]. In such a situation there could be a net area enclosed in the Force-Extension plane. This poses a puzzle because it appears that a cyclic process can extract work from the system. This puzzle is easily resolved. In completing the cycle and returning to the initial state one is in fact changing ensembles twice at the two end points. These correspond to finite free energy changes which need to be taken into account.

Let $(\zeta_1, f_1)$ and $(\zeta_2, f_2)$ be two points which lie on both isometric and isotensional force-extension curves. In our example $(\zeta_1, f_1) = (0, 0)$ and $(\zeta_2, f_2) = (1, \infty)$. Let us suppose that we go from $(\zeta_1, f_1)$ to $(\zeta_2, f_2)$ in the isotensional ensemble and return via the isometric
ensemble. We find that

\[ G(f_2) - G(f_1) = \int_{f_1}^{f_2} \frac{\partial G}{\partial f} df = - \int_{f_1}^{f_2} < \zeta > df. \quad (7) \]

Similarly,

\[ F(\zeta_2) - F(\zeta_1) = \int_{\zeta_1}^{\zeta_2} \frac{\partial F}{\partial \zeta} d\zeta = \int_{\zeta_1}^{\zeta_2} < f > d\zeta. \quad (8) \]

The area enclosed between the two curves is given by

\[ W = \int_{\zeta_1}^{\zeta_2} f d < \zeta > - \int_{\zeta_1}^{\zeta_2} < f > d\zeta \]

which can be rewritten as

\[ W = \int_{\zeta_1}^{\zeta_2} d(f < \zeta >) - \int_{f_1}^{f_2} < \zeta > df - \int_{\zeta_1}^{\zeta_2} < f > d\zeta \]

\[ = f_2 \zeta_2 - f_1 \zeta_1 + G(f_2) - G(f_1) - F(\zeta_2) + F(\zeta_1) \]

\[ = [f \zeta + G(f) - F(\zeta)]_1^2 = [\tilde{F}(\zeta) - F(\zeta)]_1^2 \]

\[ = \Delta F^{tr}(\zeta)_1^2 \]

where we define \( \Delta F^{tr}(\zeta) \) as the free energy of transition. \( \Delta F^{tr}(\zeta) \) is the difference between \( \tilde{F}(\zeta) = f \zeta + G(f) \), the Legendre transform of \( G(f) \) and the Helmholtz free energy \( F(\zeta) \). Since these are not equal (except in the limit of long polymers) the free energy of transition between ensembles must be considered in order that the total free energy change in a cyclic process vanishes.

In order to understand this issue more explicitly we consider corrections to the saddle point approximation which is valid in the long polymer limit. Let us expand \( \phi(\zeta) = \mathcal{F}(\zeta) - f \zeta \), the argument of the exponential appearing on the right hand side of Eq. (4) around the saddle point value \( \zeta = \zeta^* \) (which dominates the integral in the long polymer limit) and retain terms up to second order in the fluctuations about the saddle point value:

\[ \phi(\zeta) = \phi(\zeta^*) + \frac{1}{2} \phi^{''}(\zeta)|_{\zeta=\zeta^*}(\zeta - \zeta^*)^2. \]

If we plug in this expansion in Eq. (4) and identify \( \tilde{Z}(f, \beta) \) with \( e^{-\beta G(f)} \) we arrive at the following equation:
\[ G(f) = [F(\zeta_{*}) - f(\zeta_{*})] + \frac{1}{2\beta}lnF''(\zeta_{*}) \]  

(10)

The free energy due to fluctuations around the saddle point value is \(\frac{1}{2\beta}lnF''(\zeta_{*})\). Notice that in the long polymer limit of \(\beta \to \infty\), this term vanishes. For finite \(\beta\), this nonzero contribution to the free energy accounts for the transition between the constant extension ensemble and the constant force ensemble. In going from a soft trap to a stiff trap work is done on the bead by the trap. Similarly in going from a stiff trap to a soft trap work is extracted from the bead by the trap. The net work done is the difference between the work done at the two ends of the force-extension curves in switching ensembles. This net work exactly cancels out the nonzero area enclosed in the force-extension plane.

V “First Order Phase Transition” and the Thermodynamic Limit in Semiflexible Polymers

In Sec. II we considered the bead to be in a potential which was soft in the \(x\) and \(y\) directions. Let us now consider what happens when the trap is stiff in all three directions (\(A\) as well as \(C\) in Eq. (1) are large) and the vector position of the bead is constrained to be at \((x_0, y_0, z_0)\). Let \(Q(\vec{r})\) be the number of polymer configurations which start at the origin and end in the volume element \(d\vec{r}\) centered at \(\vec{r}\) [11,18]. \(Q(\vec{r})\) is related to \(P(z)\) via the equation

\[ P(z) = \int d\vec{r}Q(\vec{r})\delta(r_3 - z) \]  

(11)

which in words, means that \(P(z)\) is obtained by integrating \(Q(\vec{r})\) over a plane of constant \(z\) [11]. The distribution \(Q(\vec{r})\) was studied in [18] where it was noticed that in an intermediate range (around 3.8) of \(\beta\) the free energy \(A(\vec{r}) = \frac{1}{\beta}logQ(\vec{r})\) had multiple minima. For a fixed contour length as one varies \(\beta\) by tuning the persistence length \(L_P\) one finds a competition between flexible and rigid phases of the polymer for intermediate values of \(\beta\). Thus the polymer undergoes a flexible to rigid “first order phase transition” via a two peaked profile of \(Q(\vec{r})\). This leads to a curious force-extension relation. As one pulls the bead, the restoring force at first increases, then decreases to zero and then goes negative and becomes a destabilizing force. The molecule is unstable and goes to a new extended state.
The words “first order phase transition” above were in quotes as a finite system does not show phase transitions. If one takes the thermodynamic limit by taking the length of the polymer to infinity (\(\beta \to \infty\)) one loses the multiple minima structure which is present only in a small range of \(\beta\) around 3.8. Is it possible for this phase transition to survive the thermodynamic limit? As we will see below, this is indeed possible provided one takes the replica thermodynamic limit. We take \(N\) replicas of the molecule with fixed \(\beta\) and let \(N\) tend to \(\infty\). Consider \(N\) identical polymers with \(\beta = \frac{L}{L_p}\) fixed, their two ends anchored to flat surfaces \(S_1\) and \(S_2\) (See Fig. 2). One could realize the above arrangement by using (i) two planar arrays of optical traps or (ii) by introducing suitably synthesized supramolecular lamellar structures. The anchoring is such that the tangent vectors to the molecule at the fixed ends are free to swivel. If one applies a force \(F\) to pull \(S_1\) and \(S_2\) apart the \(N\) molecules are also stretched. We consider the molecules to be well separated so that they can be regarded as independent. One could now look at the mean force \(F\) needed to maintain the separation \(r\). It is easily seen that the force is proportional to \(N\) and also the mean square fluctuation \(<(\Delta F)^2>\) in the force is proportional to \(N\). This is because the mean force and its variance are respectively the first and second derivatives of the free energy, which being an extensive quantity is proportional to \(N\). It follows that as \(N\) goes to infinity, the fluctuations \((\Delta F/F)\) in \(F\) die out as \(1/\sqrt{N}\). We can now regard the mean force \(F\) as a control parameter (i.e. consider a constant \(F\) ensemble) and observe that if we tune the applied force \(F\), there is a discontinuous change in the separation \(r\) between the two sets of optical traps signalling a first order phase transition with the inter-trap separation \(r\) as the order parameter. Thus, in the replica way of taking the thermodynamic limit the double humped form of the distribution function \(Q(\vec{r})\) results in a true first order phase transition.
VI Discussion:

In this paper we point out the importance of considering the free energy of transition in going between the Helmholtz and the Gibbs ensembles in the context of single molecule force-extension measurements. We also study two distinct ways of taking the thermodynamic limit (i) by letting the length of the polymer tend to infinity (i.e. $\beta \to \infty$) and (ii) by considering replicas. In particular we notice that the flexible to rigid transition mentioned in Sec. V for a single semiflexible polymer survives the replica thermodynamic limit. In contrast, this feature disappears in the usual thermodynamic limit of $\beta \to \infty$.

The non-monotonic behavior of $Q(\vec{r})$ is an intriguing feature of molecular elasticity. It was noticed in recent simulations [18] and subsequently in a semi-analytical treatment [11]. It was also commented on in a recent paper [19]. This double humped form of $Q(\vec{r})$ has gained renewed interest in the context of cyclization of polymers and its significance to gene regulation [20,21].

We note that this remarkable qualitatively distinctive feature is special to the isometric ensemble (fixed vector end-to-end separation) and is not shared by other ensembles. In other ensembles one can argue generally that such non-monotonic behavior cannot occur. For example, the conjugate distribution $\tilde{P}(\vec{F}) = \int Q(\vec{r})e^{\vec{F} \cdot \vec{r}} d\vec{r}$ is monotonic. This follows
from noticing that the $3 \times 3$ matrix
\[ \frac{\partial \ln \tilde{P}}{\partial F_i \partial F_j} = \langle (r_i - \langle r_i \rangle)(r_j - \langle r_j \rangle) \rangle \tag{12} \]
is positive definite. Such arguments do not apply to $Q(\vec{r})$ since there is no analogous formula to Eq. (4) in the conjugate distribution. Indeed, if there were, a double humped form could not appear in $Q(\vec{r})$, for one could express the second derivative of $Q(\vec{r})$ as the variance of the force. Can $P(z)$ show non-monotonic behavior? The answer is no, for it has been shown in [11] that
\[ \frac{-2}{z} \frac{dP}{dz} = Q(z) \]
Since $Q(\vec{r})$ is a probability density and therefore non-negative, it follows that $\frac{dP}{dz} \leq 0$ for $z > 0$, thus ruling out multiple peaks in $P(z)$. This argument which rules out multiple peaks in $P(z)$ is a new observation. Note that a $P(z)$ measurement differs from $Q(\vec{r})$ only in the transverse stiffness $A$ of the trap. By tuning $A$ we can permit fluctuations in the transverse direction and therefore destroy the phase transition present in the stiff $A$ limit. One would expect to see a critical stiffness $A = A_c$ below which the phase transition is destroyed. Alternately, one could tune the mean force and expect to see the phase transition vanishing below a critical mean force $F = F_c$ for a fixed value of $\beta$ in the intermediate range of $\beta$. We emphasize that the non-monotonic features of $Q(\vec{r})$ in the semiflexible range $\beta \approx 3.8$ are predictions of the WLC model which can be tested against experiments. A single molecule with its ends confined in optical traps is expected to show this flexible to rigid transition. The effect can be dramatic however, if a large number of molecules co-operatively show such a transition. One could attach the ends of a collection of semiflexible polymers to supramolecular layers [22] and detect the flexible to rigid transition signalled by a change in the inter-layer separation via a suitable probe. It may be possible to exploit this dramatic transition from flexible to rigid behavior in technological applications.

If one considers the replica thermodynamic limit of a semiflexible polymer one sees that force-extension curves continue to remain distinct in the Helmholtz and the Gibbs ensembles. So while interpreting a force-extension curve obtained for a collection of semiflexible
polymers suspended between two arrays of traps, one needs to know if the curve is obtained using the soft trap setup or a stiff trap setup. However, for $\beta \to \infty$, which is the usual thermodynamic limit, the two ensembles give rise to the same force-extension curve. This observation is consistent with the fact that inequivalence of ensembles can survive at the thermodynamic limit for systems with long range interactions [7,8,23]. In the context of semiflexible polymers, the persistence length $L_P$ plays the role of the range of interactions.

There has been some work [24] on the thermodynamics of particle systems in the presence of external macroscopic fields in classical and quantal contexts. In these papers the authors have dealt with the macroscopic limit of the definition of pressure which is analogous to the thermodynamic limit of the definition of force in our work. In particular the authors of Ref. [24] discuss the connection between the values of the pressure defined by two different thermodynamic limit procedures: in the first, the system is confined successively in a sequence of boxes which grows to fill up the whole space. In the second, the system is in an external potential similar to the present context. In the case of a semiflexible polymer the force corresponding to a given extension is the same in the Gibbs and Helmholtz ensembles only in the thermodynamic limit of $\beta \to \infty$. This is analogous to the second procedure of taking the thermodynamic limit given in [24], where they recover the thermodynamic notion of pressure from an underlying microscopic definition when they let the scale factor go to infinity in the macroscopic limit. The results of this paper are therefore, consistent with the general treatment in [24].

The fluctuation effects mentioned here also have some biological significance. In particular, the process of gene regulation involves interaction between parts of a DNA molecule which are about less than one persistence length apart ($\approx 34nm$) [21]. Over such short segments of the DNA fluctuation effects would be significant. The replica thermodynamic limit would also play a role in the concrete biological context of a network of actin filaments forming the cytoskeletal structure.

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