

Stretching a macromolecule in an atomic force microscope: Statistical mechanical analysis

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We formulate the proper statistical mechanics to describe the stretching of a macromolecule under a force provided by the cantilever of an atomic force microscope. In the limit of a soft cantilever the generalized ensemble of the coupled molecule/cantilever system reduces to the Gibbs ensemble for an isolated molecule subject to a constant force in which the extension is fluctuating. For a stiff cantilever we obtain the Helmholtz ensemble for an isolated molecule held at a fixed extension with the force fluctuating. Numerical examples are given for poly (ethylene glycol) chains.

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A series of papers have reported the measurements of the mechanical properties of single macromolecules with the atomic force microscope (AFM) [1–8]. A macromolecule is anchored on the surface of a substrate, and the functionalized tip of an AFM cantilever picks up the molecule somewhere along its chain. By moving the cantilever, the molecule is stretched by the elastic force of the deflecting cantilever. Thus one obtains the mechanical response of the macromolecule in the form of the force-extension curve.

The force/extension relation or, in thermodynamic terms, the mechanical equation of state, can be measured and calculated under different boundary conditions: (i) One can fix the length of the macromolecule and measure the force necessary to maintain this length; this suggests doing the statistical mechanics in the isothermal–isochoric or Helmholtz ensemble in which the length is a control variable and the average force and its fluctuations are calculated by differentiation. (ii) One can apply a given force and measure the resultant extension of the molecule; this suggests doing the statistical mechanics in the isothermal–isobaric or Gibbs ensemble in which the force is a control variable, and the length and its fluctuations are calculated by differentiation [9,10].

Because different ensembles in statistical mechanics are only equivalent for thermodynamically large systems but not for small systems in which fluctuations are non-negligible, it is important to formulate the right statistical mechanics for the stretching of a macromolecule in an AFM experiment to allow interpretation of the experimental data and maximal extraction of information. The question to be answered is which of the two thermodynamically conjugate variables, force and extension, is held constant and which is the fluctuating response. We show that both situations can be realized by changing the force constant of the cantilever. So far experiments were done (approximately) under (ii), mainly for reasons of sensitivity. Recently a first principles theory was developed using both Gibbs and Helmholtz ensembles [11]. Applied to the stretching of poly (ethylene glycol), both in hexadecane and in water, quantitative agreement [12] was achieved with the experimental results [6], based on the Gibbs ensemble.

A schematic of the experimental setup is shown in Fig. 1. In the absence of contact between the cantilever tip and the macromolecule the tip would be at a distance D from the

surface where the macromolecule is anchored. When the tip is attached to the macromolecule the latter is stretched to an end-to-end length L_m and the tip is deflected by a distance L_c such that $D=L_m+L_c$. Whereas L_m is always positive, L_c can have either sign. In the experiment the distance D is adjusted and the resulting deflection, L_c , of the tip is measured optically. The force is calculated assuming, in the simplest case, Hooke's law $F=k_c L_c$; the extension, L_m , of the macromolecule is $L_m=D-L_c$. Obviously L_m undergoes thermal fluctuations and consequently F and L_c do also.

We treat the tip (cantilever) and the macromolecule as two coupled subsystems whose lengths are unknown and to be measured and calculated. The experimentally controlled variables are the distance D and the temperature T . The microstates of the system are those of the two subsystems for various lengths and internal excitations. We introduce canonical configurational partition functions of the two decoupled subsystems for given lengths, $Z_m(T, L_m)$ and $Z_c(T, L_c)$, where the subscripts refer to the macromolecule (m) and the cantilever (c). Coupling the two subsystems together allows the total system to sample all lengths L_m and L_c . Although the structure and the internal vibrational excitation spectrum of the macromolecule (more precisely of the relevant conformers of the macromolecule) as a function of its length must be calculated from quantum mechanics its coupling to the cantilever can be described adequately by classical statistical mechanics because it involves only its center of mass motion. We can therefore write for the system partition function

$$Z_{\text{system}}(T, D) = \lambda_m^{-1} \int_0^{\infty} dL_m Z_m(T, L_m) Z_c(T, D - L_m). \quad (1)$$

The thermal wavelength $\lambda_m = h/(2\pi\mu k_B T)^{1/2}$ enters via the integration over the momentum, p , of the center of mass motion of the macromolecule and of the cantilever (of reduced mass μ). Strictly speaking, L_m is the z component of a vector with the z direction along D . To restrict the cantilever to exert only stretching forces on the molecule, we could impose an upper integration limit D in (1). This would apply to long polymer chains that can easily curl up. However, short chains may resist compression, the cantilever must be allowed to bend away from the macromolecule, and the up-

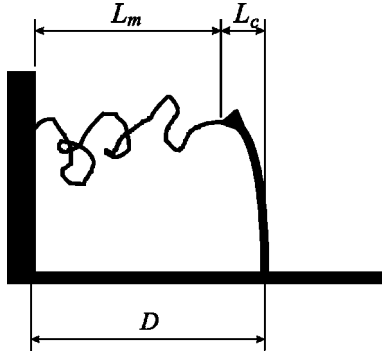


FIG. 1. Schematic of an AFM experiment to measure the force-extension curve of a macromolecule.

per limit in (1) can be much larger than D ; infinity for simplicity. From (1) we get the Helmholtz free energy of the coupled system from which we get the average force on the system by differentiation with respect to D ,

$$\bar{F}(T, D) = k_B T (\partial \ln Z_{\text{system}} / \partial D) |_T. \quad (2)$$

Because the coupled macromolecule/cantilever system is in internal equilibrium this is also the force with which the cantilever acts on the macromolecule and vice versa. The average length of the macromolecule is

$$\bar{L}_m(T, D) = \frac{\int_0^\infty dL_m L_m Z_m(T, L_m) Z_c(T, D - L_m)}{\int_0^\infty dL_m Z_m(T, L_m) Z_c(T, D - L_m)} \quad (3)$$

and the deflection of the cantilever is $\bar{L}_c = D - \bar{L}_m$. We get the force/extension curve of the macromolecule, i.e., $\bar{L}_m(T, \bar{F})$, by solving (2) and (3) simultaneously for a given temperature and varying distances D . Its explicit form obviously depends on both the intrinsic properties of the macromolecule and of the cantilever.

To make closer contact with the AFM experiment we specify the cantilever to be well approximated by a harmonic spring with spring constant k_c and canonical partition function $Z_c(T, L_c) = \exp[-\beta k_c L_c^2 / 2]$, $\beta = 1/k_B T$. Typical cantilevers used in AFM experiments have force constants varying from 1 to 100 pN/Å. We then get for the force from (2) $\bar{F}(T, D) = k_c (D - \bar{L}_m)$ where \bar{L}_m is given by (3). Thus the average force is determined by measuring the average deflection ($D - \bar{L}_m$) of the cantilever. The fluctuations of the length of the macromolecule, δL_m , induce fluctuations of the force, $\delta F = k_c \delta L_m$, so that $(\delta F / \bar{F}) = (\delta L_m / \bar{L}_m) / (D / \bar{L}_m - 1)$.

To clarify the force/extension relation we write $L_c^2 = (D - \bar{L}_m)^2 + 2(D - \bar{L}_m)(\bar{L}_m - L_m) + (\bar{L}_m - L_m)^2$, and insert this in (3) to get

$$\bar{L}_m = \frac{\int_0^\infty L_m dL_m Z_m(T, L_m) \exp[\beta \bar{F} L_m] \exp\left[-\frac{\beta k_c}{2} (\bar{L}_m - L_m)^2\right]}{\int_0^\infty dL_m Z_m(T, L_m) \exp[\beta \bar{F} L_m] \exp\left[-\frac{\beta k_c}{2} (\bar{L}_m - L_m)^2\right]}. \quad (4)$$

Together with $\bar{F}(T, D) = k_c (D - \bar{L}_m)$ this yields the force/extension curve for a macromolecule stretched by the cantilever. To measure the intrinsic properties of the macromolecule in the AFM experiment one must eliminate (minimize) the effects of the cantilever. This can be done by judicious choices of the cantilever properties, namely either “very soft” or “very stiff” cantilevers.

Soft cantilever: We take the limits $k_c \rightarrow 0$, $D \rightarrow \infty$, with $k_c D$ finite (for a finite force), which reduces (4) to

$$\bar{L}_m \approx \frac{\int_0^\infty L_m dL_m Z_m(T, L_m) \exp[\beta \bar{F} L_m]}{\int_0^\infty dL_m Z_m(T, L_m) \exp[\beta \bar{F} L_m]}.$$

This is the expression for the average length in the Gibbs (or isothermal–isobaric) ensemble of an isolated macromolecule to which an external force is applied whose origin is not explicitly identified, i.e., from a Gibbs partition function [13] $Z_m^{\text{(Gibbs)}}(T, F) = \lambda_m^{-1} \int_0^\infty dL_m Z_m(T, L_m) \exp[\beta F L_m]$ and

Gibbs potential $g(T, F) = -k_B T \ln Z_m^{\text{(Gibbs)}}(T, F)$ from which the average length follows by differentiation with respect to the force F . The only difference is that employing the Gibbs ensemble one assumes that the external force is experimentally controlled and thus does not fluctuate. One can see this explicitly: The soft cantilever limit implies that $D / \bar{L}_m \rightarrow \infty$ so that the relative force fluctuations, $\delta F / \bar{F}$, become arbitrarily small. Thus the criterion for a “very soft” cantilever is that $D / \bar{L}_m \gg 1$. This is indeed the case in the series of experiments done by Gaub and co-workers [6]. There is of course a balance to be struck in the sense that the noise in the cantilever increases with its softness.

Stiff cantilever: We start from the system partition function (1) and note that in the limit $k_c \rightarrow \infty$, the cantilever (harmonic) partition function approaches a delta function so that (1) factorizes $Z_{\text{system}}(T, D) \rightarrow Z_m(T, D) (k_B T / \hbar \omega_c)$ where $\omega_c = (k_c / \mu)^{1/2}$. Thus the free energy separates into two terms, the first being the Helmholtz free energy of the isolated macromolecule of specified length D , and the second arising from the cantilever. In this limit the force is obtained

by differentiation of the Helmholtz free energy of an isolated macromolecule of length D . That this is indeed the case can be demonstrated by observing that the stiff cantilever limit implies that $D/\bar{L}_m \rightarrow 1^+$. Rewriting the fluctuations as $(\delta L_m/\bar{L}_m) = (D/\bar{L}_m - 1) \times (\delta F/\bar{F})$, we see that in this limit the length fluctuations are reduced to zero which is the prerequisite for the use of the Helmholtz ensemble for the (isolated) macromolecule. Our criterion for a “very stiff” cantilever is therefore that $(D/\bar{L}_m - 1) \ll 1$. This limit is experimentally more difficult to achieve than the soft limit in that the deflection of a stiff cantilever is obviously very small so that its sensitivity becomes poor. Yet, as we will see in the numerical examples, this limit is physically also interesting and thus worth pursuing experimentally. One should keep in mind that once theory has produced a quantitative explanation of the soft cantilever experiments it is an easy task to calculate what one would expect for a stiff cantilever.

To calculate the force/extension curve for a given macromolecule we must first obtain its canonical partition function, $Z_m(T, L_m)$, for a fixed length L_m . For a specific force constant, k_c of the cantilever we follow the procedure in an AFM experiment, and select a range of D settings and calculate the length $\bar{L}_m(T, D)$ which, together with $\bar{F}(T, D) = k_c(D - \bar{L}_m)$ yields the self-consistent force $\bar{F} = \bar{F}(T, \bar{L}_m)$. How much this curve deviates from the force/extension curve of an *isolated* macromolecule depends on the force constant of the cantilever. We demonstrate this for a chain of poly (ethylene glycol) (PEG) with 21 subunits [(EG)₂₁].

We have recently presented a theoretical description [11,12] of the force measurements reported by Oesterhelt *et al.* [6] on individual poly (ethylene glycol) chains (PEG) in different solvents, i.e., PBS buffer or hexadecane. In our first principles theory we (i) calculated the energy spectrum (or the density of states) for short PEG chains from quantum mechanics [14], and (ii) used the Gibbs ensemble to derive the force/extension curve. One can also use these results of *ab initio* calculations for short polymer molecules to construct an interacting Ising-type chain model valid for any length of the polymer. Details of such calculations and all the parameters of the chain model are given elsewhere [15]. Using this interacting chain model we have calculated the force extension curves for PEG with 21 EG subunits for various force constants of the cantilever.

In Fig. 2 we show force/extension curves for (EG)₂₁ as stretched by cantilevers for a range of spring constants. In the top panel we show the force/extension curves for soft cantilevers, i.e., for spring constants less than 1 pN/Å. These curves are, to within a fraction of a percent, equal to those of an isolated macromolecule stretched by an external force as calculated with the Gibbs ensemble as discussed above. In the same panel we can also see that lowering the temperature sharpens up some features in the force/extension curve. For shorter chains, which have far fewer conformers and thus are much less flexible, e.g. (EG)₃, the force is negative at end-to-end lengths of less than about 2 Å per monomer.

We have also plotted the settings of D necessary to measure this curve with a cantilever of 1 pN/Å: Not surprisingly this weak cantilever needs substantial deflection (about three

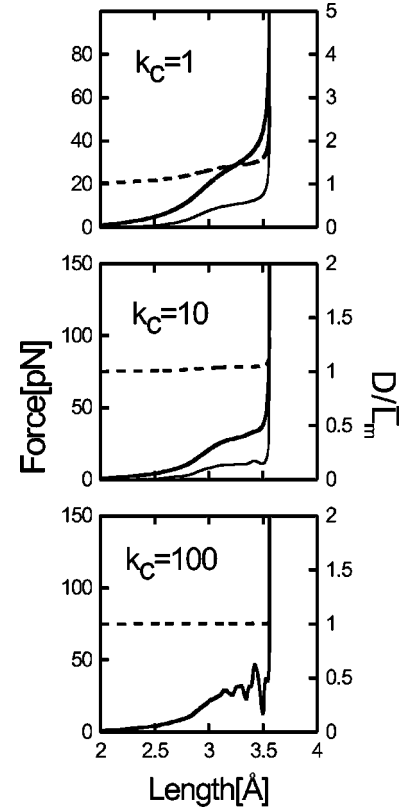


FIG. 2. Force/extension curves for (EG)₂₁ as “measured” in an AFM experiment with cantilevers of different force constants k_c in piconewton per angstrom as indicated at 300 K (thick solid lines) and at 100 K (thin solid lines). Also shown as dashed lines is the ratio of the position D of the cantilever to the length \bar{L}_m of the macromolecule (right scale).

times the length of the macromolecule at maximum extension) to produce forces of the order of 400 pN. For a softer cantilever with $k_c = 0.1$ pN/Å the ratio D/\bar{L}_m would go up to 10, and for $k_c = 10$ pN/Å, center panel of Fig. 2, the setting D is only 10–20% larger than \bar{L}_m for the largest extension. Large deflection of course also implies good sensitivity of the cantilever provided its fluctuations remain manageable. These numbers agree remarkably well with the settings in the experiment by Oesterhelt *et al.* [6]. Remarkable is the change in the distance D needed for the measurement of the force/extension curves as one goes from a long to a short chain, and also from stiffer to softer cantilevers, e.g., for the short chain of only 3 EG subunits and $k_c = 10$ pN/Å, we need $D/\bar{L}_m \approx 20$ for the largest extension.

In the center panel of Fig. 2 we show the force/extension curves (again for 300 and 100 K) for a larger force constant, showing already some modification from the soft cantilever (i.e., Gibbsian) limit. We emphasize, in an AFM experiment with intermediate strength cantilevers [for (EG)₂₁ and (EG)₃ this is the range from 1 to 10 and from 10² to 10³ pN/Å, respectively] the measured force/extension curve would not be that of an isolated macromolecule. It would take considerable effort (such as our theoretical approach presented here) to disentangle the features arising from the macromolecule itself and those from its coupling to the cantilever. For

such cantilevers the D settings needed are only slightly larger than the end-to-end length of the stretched macromolecule, i.e., the deflections of the cantilever are becoming marginal.

In the bottom panel of Fig. 2 we show a force/extension curve at 300 K as calculated for a very stiff cantilever. We are approaching the boundary conditions of the Helmholtz ensemble for an isolated macromolecule, held at fixed length D . The force starts to oscillate between attraction and repulsion as new conformers contribute. This is most pronounced at the largest extension (and also at lower temperatures) where the number of conformers available becomes rather sparse. Note also that, with the cantilever much stiffer than the molecule itself (except for the longest extension possible, where within an interacting chain model the stiffness is infinite), the setting D is only marginally larger than the resulting extension of the molecule, \bar{L}_m . The curves in the top and bottom panels, albeit measured under different limits for the cantilever, contain information solely about the intrinsic properties of the isolated macromolecule.

To see the physical significance of the different boundary conditions, i.e., fixed length (Helmholtz) vs fixed force (Gibbs), we illustrate these two situations schematically for three conformers in Fig. 3. At fixed length we take the derivatives of the three potential energy curves and add these different forces with their respective Boltzmann factors. At fixed force we sample those points on the potential energy curves at different lengths where the derivatives are the same. Ideally, in an experiment, one would switch to cantilevers with larger force constants for the measurement of the high-force regime to minimize the cantilever extension $D - \bar{L}_m$ and reduce its fluctuations.

In this paper we have set up the theory to describe the stretching of a macromolecule by a cantilever in an AFM experiment. We have shown that for intermediate cantilever force constants the elastic and energetic properties of both the macromolecule and the cantilever contribute to the force/extension curve in a convoluted way. However, for soft cantilevers a situation can be achieved in which the effect of the cantilever on the force/extension curve becomes negligible,

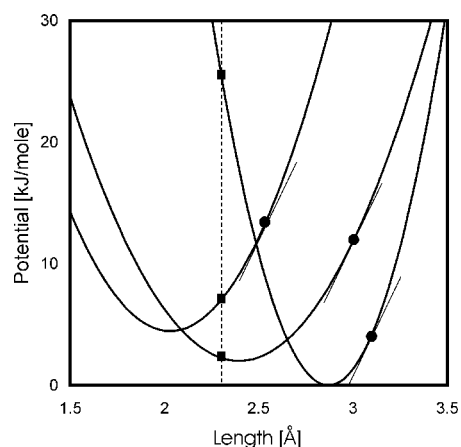


FIG. 3. Schematic to illustrate different boundary conditions. Working at fixed length (Helmholtz ensemble), one samples different forces, i.e., the derivatives of the potential energy curves at that length (squares). Working at fixed force, one samples the same slope on the energy curves, albeit at different lengths (circles).

and the latter can be calculated using the Gibbs ensemble for an isolated molecule as we have done in our previous work [11,12]. On the other hand, for very stiff cantilevers the force/extension curve resembles that which one would obtain from a calculation in the Helmholtz ensemble of the isolated macromolecule. These two ensembles do not produce the same mechanical equation of state (i.e., force/extension curve) as they would for a macroscopically large system, because polymer molecules even with several hundred monomer units are still substantially influenced by fluctuations, in particular in the force needed to stretch them.

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