Thermodynamics for Single-Molecule Stretching Experiments

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We show how to construct nonequilibrium thermodynamics for systems too small to be considered thermodynamically in a traditional sense. Through the use of a nonequilibrium ensemble of many replicas of the system which can be viewed as a large thermodynamic system, we discuss the validity of nonequilibrium thermodynamics relations and analyze the nature of dissipation in small systems through the entropy production rate. We show in particular that the Gibbs equation, when formulated in terms of average values of the extensive quantities, is still valid, whereas the Gibbs−Duhem equation differs from the equation obtained for large systems due to the lack of the thermodynamic limit. Single-molecule stretching experiments are interpreted under the prism of this theory. The potentials of mean force and mean position, now introduced in these experiments in substitution of the thermodynamic potentials, correspond respectively to our Helmholtz and Gibbs energies. These results show that a thermodynamic formalism can indeed be applied at the single-molecule level.

1. Introduction

Thermodynamics[1] can only be applied to systems with an infinite number of particles distributed in an infinite volume with a constant density, a situation referred to as the thermodynamic limit. Under these circumstances, one may adopt a coarse description of the system ensuring the existence of extensive quantities and justifying the absence of fluctuations. The thermodynamic limit[2] can be asymptotically approximated through statistical mechanics, and the results from different statistical ensembles converge. It is then said that thermodynamics deals with large-scale systems.

The lack of a thermodynamic limit has important consequences for the behavior of the system. Scaling down the size leads to a very different scenario, in which contributions to the energy of the system not present in the thermodynamic limit, such as surface energy, may show up, thereby breaking extensivity and making a normal thermodynamic treatment impossible. A typical example is a small cluster of N particles whose Gibbs energy is given by[3]

$$G = \mu N + aN^{\beta} \tag{1}$$

with μ the chemical potential, a an arbitrary function of the intensive parameters, and $\beta < 1$ an exponent. When the cluster contains a large number of particles, the second contribution becomes much smaller than the first and can be neglected. At this limit, one obtains the well-known thermodynamic relation $G \cong \mu N$ for which the chemical potential is the Gibbs energy per particle. On the other hand, for clusters containing a smaller number of particles, both contributions must be considered, which makes the Gibbs energy nonextensive. As a consequence, thermodynamics no longer applies. Without a coarse description,

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fluctuations become important and play a role in the characterization of the system.

Despite this relevant feature, a thermodynamic treatment of the small system is still possible. The way to proceed was proposed by Hill[3] for systems at equilibrium and was subsequently applied to different situations such as biochemical cycle kinetics,[4,5] open metastable systems,[6] entropy−enthalpy compensation effects,[7,8] and critical behavior of ferromagnets.[9] It was later referred to as nanothermodynamics.[10] Given a small system, the ensemble of many of its replicas becomes a large system having the usual thermodynamic behavior. To restore the extensivity of the Gibbs energy,[1] one can express it as

$$G = \hat{\mu} N \tag{2}$$

where the chemical potential $\hat{\mu}$, according to eq 1, is given by[3]

$$\hat{\mu} = \mu + aN^{\beta-1} \tag{3}$$

Both chemical potentials thus coincide at the thermodynamic limit. It can also be shown that thermodynamic quantities defined for different ensembles may not be equivalent.[3]

Thermodynamics of small systems has been formulated at an equilibrium state.[3] In many experimental situations, however, instead of being in a quiescent state, the system evolves in time adopting different nonequilibrium configurations. This situation is commonly found in kinetic processes such as nucleation[11] and growth[12] of small clusters, in noncovalent association between proteins,[13] and in active transport through biological membranes.[14,15] It is our purpose in this paper to develop the nonequilibrium thermodynamics of small systems offering an interpretation of the nonequilibrium thermodynamics concepts as well as identifying the role played by dissipation.

The paper is organized as follows. Section 2 presents the main ideas of Hill's thermodynamics of small systems. In section 3, we proceed with the study of small systems outside equilibrium by introducing the nonequilibrium ensemble of replicas. The

nonequilibrium thermodynamics relations and the entropy production of small systems are obtained in section 4. In section 5, we apply the nonequilibrium thermodynamics of small systems developed to give a thermodynamic interpretation of single-molecule experiments. Finally, in the Conclusions section, we summarize our main results and discuss perspectives of the theory presented.

2. The Thermodynamic System of Replicas

A small system can be made large by constructing an ensemble of identical, independent replicas. This procedure ensures the validity of thermodynamic relations in the ensemble.[3] Consider a group with K number of these replicas with total energy $U_{tot} = K\bar{U}$, entropy $S_{tot} = KS$, and volume $V_{tot} = K\bar{V}$. Here, $\bar{U}$ is the average internal energy, S is the entropy, and $\bar{V}$ the average volume per replica. The total quantities are extensive by definition. According to the first law, we can change the energy by adding heat or work to the ensemble

$$dU_{tot} = dQ + dW \qquad (4)$$

At reversible conditions, the added heat Q_{rev} follows from the definition of the entropy

$$dS_{tot} = \frac{dQ_{rev}}{T} \qquad (5)$$

whereas the irreversible heat enters into the entropy production.

Let us assume that the system has a constant number of particles N and is in contact with pressure and heat reservoirs, keeping its pressure p and temperature T constant. Changes in the energy of the ensemble are given by

$$dU_{tot} = T\,dS_{tot} - p\,dV_{tot} + \mu K\,dN + X\,dK \qquad (6)$$

Here, μ is the chemical potential of a large system of N particles, with $K = 1$. The last term accounts for the energy raise by adding more replicas to the ensemble and also represents an increase of entropy, since the total number of particles of the ensemble can spread over a larger number of replicas. This term is not present in a large system for which $K = 1$.

The energy of the ensemble follows by integrating this expression, keeping N, p, and T constant

$$U_{tot} = TS_{tot} - pV_{tot} + XK \qquad (7)$$

and the average energy is in light of the relation between total and average values given by

$$\bar{U} = TS - p\bar{V} + G \qquad (8)$$

where we have identified X with the Gibbs energy G. Extensivity can be restored by expressing this quantity as

$$G = \hat{\mu}N \qquad (9)$$

with $\hat{\mu}$ the corresponding chemical potential. Expression 8 can also be written as

$$\bar{U} = TS - p\bar{V} + \mu N + (\hat{\mu} - \mu)N \qquad (10)$$

This equation does not correspond to the one derived for a thermodynamic system. Deviations from the large-system behavior are due to the presence of the term $(\hat{\mu} - \mu)$ vanishing at the thermodynamic limit in which $G = \mu N$. Variations of the mean energy value are then given by

$$d\bar{U} = T\,dS - p\,d\bar{V} + \mu\,dN \qquad (11)$$

showing that mean value variations obey the same thermodynamic relation as for a large system.

A similar analysis can be performed for constant N, V, and T. One obtains in this case

$$dU_{tot} = T\,dS_{tot} - pK\,dV + \mu K\,dN + A\,dK \qquad (12)$$

where $A = \bar{U} - TS$ is the Helmholtz energy which differs from $-pV + \mu N$, the value encountered in thermodynamics. The Gibbs equation becomes

$$d\bar{U} = T\,dS - p\,dV + \mu\,dN \qquad (13)$$

The fact that the spreading of particles of the ensemble over the different replicas depends on the conditions imposed on the system makes the thermodynamic quantities for small systems different when these conditions change. Thermodynamic relations thus depend on the ensemble used and consequently on the measurement techniques for the experiments.

3. The Statistical Ensemble of Nonequilibrium Replicas

Application of external forces or gradients move the system from the reference state, driving it through different nonequilibrium configurations described by the set of coordinates x and characterized by the probability distribution function $P(x, t)$. The coordinates may, for example, represent the number of particles of a cluster or the configurations of a polymer or may in general be a reaction coordinate or an order parameter characterizing the state of the system. The ensemble of K replicas becomes under the presence of the external driving force a nonequilibrium ensemble whose evolution in time is described by the probability distribution function $P(\Gamma, t)$, with $\Gamma = (x_1, .., x_K)$ a point in the phase space of the ensemble. Since the elements of the ensemble are by definition independent systems, we have $P(\Gamma, t) = P(x_1) ... P(x_K)$. The time-dependent entropy of the ensemble is, according to the Gibbs entropy postulate, given by

$$S_{tot}(t) = S_{tot,0} - k_B \int P(\Gamma, t) \ln \frac{P(\Gamma, t)}{P_0(\Gamma)}\,d\Gamma \qquad (14)$$

where $S_{tot,0}$ is the entropy of the ensemble in the reference state, $P_0(\Gamma)$ the probability distribution of the ensemble in that state, and k_B the Boltzmann constant. Using the fact that the replicas are independent, we can rewrite this equation as

$$S_{tot}(t) = S_{tot,0} - K\left[k_B \int P(x, t) \ln \frac{P(x, t)}{P_0(x)}\,dx\right] \qquad (15)$$

where $P_0(x)$ denotes the probability distribution of the system at the reference state. The term within brackets of this expression represents the change in the entropy of one replica system with respect to its value at the reference state. Our construction then preserves the additive nature of the nonequilibrium entropy.

4. Nonequilibrium Thermodynamics of Small Systems

On the basis of the statistical expression for the entropy of the ensemble found in the previous section, we will proceed to formulate the nonequilibrium thermodynamics of small systems by deriving the relations common in nonequilibrium thermodynamics and by giving the expression for the entropy production.

4.1. Thermodynamic Relations. We will assume that, in the reference state, the system is at local equilibrium. Changes in the nonequilibrium entropy during the evolution in time of the

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ensemble follow from the expression obtained for the nonequilibrium entropy. We have

$$dS_{tot}(t) = dS_{tot,0} - K\left(k_B\delta \int P(x, t) \ln \frac{P(x, t)}{P_0(x)} dx\right) \quad (16)$$

Entropy variations in the local equilibrium state have the same form as those at equilibrium given through eq 1. Using this result in eq 6, one obtains

$$T\, dS_{tot}(t) = dU_{tot}(t) + p\, dV_{tot}(t) - N\hat{\mu}\, dK - \mu_0 K\, dN - \\ K\left[k_B T\delta \int P(x, t) \ln \frac{P(x, t)}{P_0(x)} dx\right] \quad (17)$$

where μ_0 is the chemical potential of the system at the local equilibrium state. The variations of the last term can be expressed as

$$k_B T\delta \int P(x, t) \ln \frac{P(x, t)}{P_0(x)} dx = \\ \int \mu_x(x, t)\delta P(x, t)\, dx - \int \mu_0 \delta P(x, t)\, dx \quad (18)$$

where we have defined the chemical potential along the x-coordinate as

$$\mu_x(x, t) = k_B T \ln \frac{P(x, t)}{P_0(x)} + \mu_0 \quad (19)$$

Using this last expression in eq 17 together with the normalization of the probability distribution

$$N = \int P(x, t)\, dx \rightarrow dN = \int \delta P(x, t)\, dx \quad (20)$$

one arrives at the Gibbs equation of the ensemble

$$T\, dS_{tot} = dU_{tot} + p\, dV_{tot} - N\hat{\mu}\, dK - K \int \mu_x \delta P\, dx \quad (21)$$

giving entropy changes at the local equilibrium state of the ensemble. We suppressed the time and x dependence of the variables to simplify the notation. The last term integrates all entropy changes due to variations of the configurations in x-space of the system. Using now the relations $S_{tot} = KS$, $U_{tot} = K\bar{U}$, and $V_{tot} = K\bar{V}$ and eq 8, we obtain from the Gibbs equation

$$T\, dS = d\bar{U} + p\, d\bar{V} - \int \mu_x \delta P\, dx \quad (22)$$

which shows that a Gibbs equation for small systems is valid when formulated in terms of average values. The probability distribution function is the average of the microscopic density distribution and can be expressed as $P(x, t) = \langle \delta[x - x(t)] \rangle$, where the average is taken over the possible realizations of the Langevin force, for a given initial condition. This probability is governed by a Fokker−Planck equation. which for a thermodynamic system can be derived within the framework of mesoscopic nonequilibrium thermodynamics.[16,17]

The Gibbs−Duhem equation relating variations of the intensive parameters can be obtained from eqs 8, 20, and 22

$$-S\, dT + \bar{V}\, dp - N\, d\hat{\mu} = \int (\hat{\mu} - \mu_x)\delta P\, dx \quad (23)$$

This equation differs from the one derived for large systems and tends to it at the thermodynamic limit where $\hat{\mu}$ and μ_x

coincide. The sign of the integral depends on the type of process the system undergoes.

4.2. Entropy Production. The Gibbs eq 22 and the first law of thermodynamics, formulated in terms of average values

$$d\bar{U} = dQ - p_{ext}\, d\bar{V} \quad (24)$$

where Q is the heat supplied and p_{ext} the external pressure imposed to the system, can be combined to obtain the rate of entropy change

$$\frac{dS}{dt} = \frac{1}{T}\frac{d\bar{U}}{dt} + \frac{p}{T}\frac{d\bar{V}}{dt} - \frac{1}{T}\int \mu_x \frac{\partial P}{\partial t}\, dx \quad (25)$$

This equation can be rewritten in the well-known general form

$$\frac{dS}{dt} = J_s + \sigma \quad (26)$$

where

$$J_s = \frac{1}{T_B}\frac{dQ}{dt} \quad (27)$$

represents the entropy exchange with the environment, and T_B is the temperature of the thermal bath. The second contribution is the entropy production rate

$$\sigma = \left(\frac{1}{T} - \frac{1}{T_B}\right)\frac{dQ}{dt} + \frac{p - p_{ext}}{T}\frac{d\bar{V}}{dt} - \frac{1}{T}\int J\frac{\partial \mu}{\partial x}\, dx \quad (28)$$

which has to be nonnegative due to the second law of thermodynamics. To obtain this equation, in eq 25 we have used the continuity equation for the probability distribution function

$$\frac{\partial P}{\partial t} = -\frac{\partial J}{\partial x} \quad (29)$$

with J the probability current along the x-coordinate, and then performed a partial integration neglecting boundary terms. As the probability distribution, this current also represents an average value.

The first two contributions of the entropy production originate from the interaction with the thermal bath because the temperature and the pressure of the system may not coincide with the corresponding quantities of the bath. The third contribution results from the underlying diffusion process along the x-coordinate. When this process is very fast, the system reaches a quasi-equilibrium state almost immediately. In this state, the current does not depend on the coordinate, and the last contribution becomes proportional to $J\Delta\mu$. From the entropy production, one can now infer expressions for the rates, as is usually done in nonequilibrium thermodynamics.[18]

5. Thermodynamics of Single-Molecule Measurements

Polymer stretching experiments have recently been performed to analyze the validity of thermodynamics for measurements made on single molecules, arriving at the conclusion that when the system is too small the usual thermodynamic relations are no longer valid, and a thermodynamic treatment is less useful.[19] These experiments give different results when the end-to-end distance of a single molecule is held fixed and the force fluctuates, compared to when the force is fixed and the end-to-end distance fluctuates. These differences should vanish in the thermodynamic limit of an infinite length. A single molecule is a particular case of a small system. We shall show in this

section that the theory we propose in this paper provides a thermodynamic interpretation of the experimental measurements. For this purpose, we will assume that the set of measurements performed as a function of time defines the ensemble of replicas. As indicated by the experiments, the two types of experiments must be analyzed using different environmental variables. In both cases, N and T are constant for the single molecule. There are two ensembles: one in which the applied force is constant and the other for which the end-to-end distance is constant.

5.1. Isometric Experiments. In the case in which the end-to-end distance l and the temperature T are externally controlled, energy variations in the ensemble of K replicas are, in accordance with eq 12, given by

$$dU_{tot} = T \, dS_{tot} + fK \, dl + A dK \qquad (30)$$

where the tensile applied force f is interpreted as the change in the internal energy of the ensemble with respect to the elongation of the polymer per replica, keeping the entropy and the number of replicas constant. In these experiments, one employs a single replica and measures a force which fluctuates as a function of the time. Averaging this quantity over the time is equivalent to ensemble averaging and therefore gives the f we need. Integration of this expression for a constant length leads to

$$U_{tot} = TS_{tot} + AK \qquad (31)$$

Using the definitions $U_{tot} = K\bar{U}$ and $S_{tot} = KS$ in this relation, we can identify $A = \bar{U} - TS$ with the Helmholtz energy previously defined. A is the change of the internal energy due to increasing the number of replicas by one, keeping S_{tot} and l constant. Variations of the average energy then follows from our previous expressions

$$d\bar{U} = T \, dS + f \, dl \qquad (32)$$

The Gibbs−Duhem equation can be derived from eq 32 and the definition of the Helmholtz energy, thus obtaining

$$S \, dT + dA = f \, dl \qquad (33)$$

If we now define the Helmholtz energy per unit of length

$$\hat{f} \equiv \frac{A}{l} \qquad (34)$$

we obtain the Gibbs−Duhem equation

$$-S \, dT + l \, d\hat{f} = (f - \hat{f}) \, dl \qquad (35)$$

From eq 33, it follows that

$$f = \left(\frac{\partial A}{\partial l}\right)_T \qquad (36)$$

from which one finds the expression for the Helmholtz energy

$$A(T, l) = \int_0^l f(T, l') \, dl' \qquad (37)$$

where the integration constant is equal to zero. The Helmholtz energy is therefore equal to the potential of mean force introduced by Keller et al.[19] It follows from this expression that the quantity $\hat{f}(l)$ is the average of the force $f(l)$ over the lengths between zero and l and is therefore generally different from f. This difference is in relationship to its dependence on the polymer elongation. At the thermodynamic limit of a very large l, $f \rightarrow \hat{f}$. At this limit, eq 35 becomes the usual Gibbs−Duhem equation of thermodynamics.

To obtain the entropy production, we will use the first law

$$d\bar{U} = dQ + f_{ext} \, dl \qquad (38)$$

In this equation, the first term on the right-hand side gives the heat which enters the average replica from the heat bath. The second term is the work done by an external force when one changes the length of the polymer, which is the same for all replicas in the isometric experiments. In combination with eq 32, the first law yields the entropy rate of change

$$\frac{dS}{dt} = \frac{1}{T}\frac{dQ}{dt} + \frac{1}{T}(f_{ext} - f)\frac{dl}{dt} \qquad (39)$$

Considering that the temperature of the bath is equal to that of the system, we can identify $1/T \, dQ/dt$ with the entropy flux into the system and find for the average entropy production per replica

$$\sigma = \frac{1}{T}(f_{ext} - f)\frac{dl}{dt} \qquad (40)$$

In the isometric equilibrium experiments by Keller et al.,[19] the length of the polymer is kept constant, and $f_{ext} = f$. In that case, the entropy production vanishes. If one proceeds to change f_{ext}, one can describe the system using the linear force flux relation

$$f_{ext} - f = \xi \frac{dl}{dt} \qquad (41)$$

where ξ is the friction coefficient.

5.2. Isotensional Experiment. In the case in which the applied force f and the temperature T are externally controlled, energy variations in the ensemble of K replicas are given by

$$dU_{tot} = T \, dS_{tot} + f \, dl_{tot} + G \, dK \qquad (42)$$

We define the average length of a replica by $l_{tot} = K\bar{l}$. The Gibbs energy is

$$G = \bar{U} - TS - f\bar{l} = A - f\bar{l} \qquad (43)$$

In the thermodynamic limit, $G = 0$. Following the steps indicated in the previous case, we obtain the Gibbs equation

$$d\bar{U} = T \, dS + f \, d\bar{l} \qquad (44)$$

and the Gibbs−Duhem equation

$$-S \, dT - \bar{l} \, df = dG \qquad (45)$$

from which we obtain the average elongation length

$$\bar{l} = -\left(\frac{\partial G}{\partial f}\right)_T \qquad (46)$$

In the experiment, one keeps f constant and measures l as a function of time. Averaging over time gives the ensemble average of l, which is equal to the $\bar{l}$ we use. We conclude that

$$G(T, f) = -\int_0^f \bar{l}(T, f) \, df \equiv X(T, f) \qquad (47)$$

where $X(T, f)$ is the Gibbs energy in the presence of a force which coincides with the potential of mean position introduced by Keller et al.[19] This quantity gives information about the mean elongation of the macromolecule for different values of the force.

To obtain the entropy production, we use the first law in the following way

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$$d\bar{U} = dQ + f_{\text{ext}} d\bar{l} \qquad (48)$$

Proceeding as in the previous case, we obtain the entropy production

$$\sigma = \frac{1}{T}(f_{\text{ext}} - f)\frac{d\bar{l}}{dt} \qquad (49)$$

In the isotentional equilibrium experiments by Keller et al.,[19] f_{ext} and f were kept equal and constant, while the average length of the polymer was also constant. In that case, the average entropy production vanishes. If one proceeds to change f_{ext}, one can describe the system using the linear force flux relation

$$f_{\text{ext}} - f = \xi \frac{d\bar{l}}{dt} \qquad (50)$$

Comparing this equation to eq 41, we see that, while eq 41 contains the average force f and the controlled length l, eq 50 contains the controlled force f and the average length $\bar{l}$. In the thermodynamic limit, both equations become the same.

Calculating the Gibbs energy gives generally different results depending on whether we use isometric or isotensional ensembles. As previously seen, when the force is constant, $G = A - \bar{f}\bar{l}$, whereas for a constant elongation, we can construct G from A, giving $A - \bar{f}l$. Only when the force is linear in the elongation, or equivalently when the Helmholtz energy is quadratic, will both expressions coincide. Results obtained from both of these ensembles would then be equivalent only when the force is harmonic.[19]

6. Conclusions

In this paper, we have shown how nonequilibrium thermodynamics can be constructed for the irreversible evolution of small systems. By considering a nonequilibrium ensemble of many replicas as a large, and therefore thermodynamic, system, we can define extensive variables and then use well-known thermodynamic concepts and relations to apply these to small systems. For different types of environmental variables, one can derive the equivalent Euler, Gibbs, and Gibbs−Duhem equations and the entropy production from which dissipation in small systems can be analyzed. The thermodynamic relations obtained agree at the thermodynamic limit with the ones of classical thermodynamics.

We have applied the formalism developed to interpret stretching experiments performed with a DNA molecule. It has been argued that the thermodynamic concepts and relations are less useful in these experiments and that the potentials of mean force and mean elongation should replace the Helmholtz and Gibbs energies.[19] When we proceed along the lines indicated, we can define extensive quantities and show that thermodynamics does apply on the single-molecule level. We have shown that these potentials introduced in the experiments coincide with our expressions for the Helmholtz and Gibbs energies and that the thermodynamic relations depend on the type of ensemble used. Our formalism gives the expression for the entropy production corresponding to the experimental situations discussed.

Our analysis may provide a thermodynamic basis for experiments performed on small systems which operate under nonequilibrium conditions for which the absence of the thermodynamic limit and the importance of the fluctuations may constitute relevant factors in the characterization of their equilibrium and dynamical properties. Clusters, single molecules, small pumps, and motors are cases where our theory could systematicaly be applied.

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