The unreasonable effectiveness of equilibrium theory for interpreting nonequilibrium experiments

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There has been much interest in applying the results of statistical mechanics to single molecule experiments. Recent work has highlighted nonequilibrium work-energy relations and fluctuation theorems that have an equilibriumlike (time independent) form. I give a simple heuristic example where an equilibrium result (the barometric law for colloidal particles in water) can be derived using the thermodynamically nonequilibrium behavior of a single colloidal particle falling through the water due to gravity. This description is possible because the particle, even while falling, is in mechanical equilibrium (the gravitational force equals the viscous drag force) at every instant. The results are generalized using Onsager's thermodynamic action approach for stochastic processes to derive time independent equations that hold for thermodynamically nonequilibrium (and even nonstationary) systems. These relations offer great possibilities for the rapid determination of thermodynamic parameters from single molecule experiments. © *2006 American Association of Physics Teachers.*

I. INTRODUCTION

Great advances have been made recently in the experimental study of very small (nanometer size) systems. It has become possible to follow the stretching and unfolding of a single protein pulled by an atomic force microscope (AFM) (Ref. 1) and to monitor the stepping of an individual molecular motor driven by a chemical fuel and influenced by an external force exerted by an optical trap.[2] These experiments are in many ways analogous to those devised by Galileo to test the fundamental laws of macroscopic mechanics. Unlike large spheres falling through air or rolling down inclined planes, single molecules in water are subject to very significant thermal fluctuations and to viscous drag forces so large that in almost all cases inertia (the $m\dot{v}$ term in Newton's equation) is negligible.[3] The development of a theory to describe these thermodynamically nonequilibrium single molecule experiments is of great importance, not only for fundamental understanding, but also to facilitate development of nanotechnology.

In this paper, I discuss the Onsager-Machlup[4] theory for nonequilibrium stochastic processes, of which single molecule experiments provide interesting recent examples. Because molecules in solution, even under the influence of an external force, are at every instant very close to mechanical equilibrium, that is, the viscous drag force is equal and opposite the net mechanical force, Onsager's theory is applicable despite the fact that most single macromolecule experiments are done far from thermodynamic equilibrium. Using Onsager's approach, it is simple to derive time independent equations relating the work done by the external forcing to the internal potential due to intramolecular forces in the single molecule.

To make the basic ideas clear, we first consider a very simple system, a colloid in water.

II. EQUILIBRIUM DESCRIPTION OF A COLLOIDAL SUSPENSION

The thermodynamic equilibrium distribution for colloidal particles in dilute aqueous suspension follows the familiar barometric or exponential law[5–7]

$$\frac{c_{\text{eq}}(h_j)}{c_{\text{eq}}(h_i)} = e^{-mg(h_j-h_i)/k_BT}, \tag{1}$$

where $c_{\text{eq}}(h_j)$ and $c_{\text{eq}}(h_i)$ are the equilibrium concentrations of particles at heights h_j and h_i, respectively, g is the acceleration due to gravity, k_B is Boltzmann's constant, and T is the absolute temperature. For spherical particles of radius r, the effective mass is $m=4\pi r^3(\rho_p-\rho_w)/3$, where ρ_p and ρ_w are the mass densities of the particle and water, respectively. Equation (1) is an equilibrium result that implicitly involves many particles so that the concentrations (particle densities) are well defined.

III. SINGLE PARTICLE PERSPECTIVE

We can look at colloids from the very different perspective of a single particle falling through solution (see Fig. 1). The forces acting are gravity, mg, viscous drag $\gamma v \sim \eta r v$ (where $\eta \approx 10^{-3}$ kg/(m s) is the viscosity of water), and a random thermal noise force due to the molecular movement of the water molecules. After a sufficiently long time (about $m/\gamma = 10^{-6}$ s for a micron-sized particle) the particle reaches terminal velocity $v_{\text{term}}=mg/\gamma$, where the force of gravity is balanced by the viscous drag and there is no further acceleration, that is, the particle is in mechanical equilibrium. Let the particle's height be h_i at time $t=0$ after the terminal velocity is attained. The subsequent probability density function $P(h_j,t|h_i,0) \equiv P(h,t)$ for the particle's position is[8]

$$P(h,t) = \frac{1}{\sqrt{4\pi Dt}}e^{-(h + (mg/\gamma)t)^2/4Dt}, \tag{2}$$

where $h=h_j-h_i$. Equation (2) is a solution of Fick's equation for diffusion with drift[9]

$$\frac{\partial P(h,t)}{\partial t} = D\frac{\partial^2 P(h,t)}{\partial h^2} + \frac{mg}{\gamma}\frac{\partial P(h,t)}{\partial h}, \tag{3}$$

where D is the diffusion coefficient.

Equation (2) describes a Gaussian distribution with mean position $\mu=-(mg/\gamma)t$ and variance $\sigma^2=\langle h(t)^2\rangle-\langle h(t)\rangle^2$
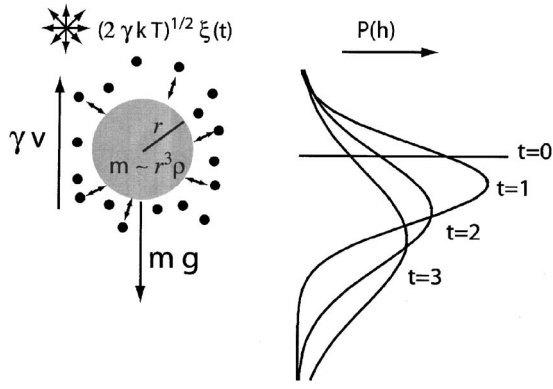
Fig. 1. A single colloidal particle falling in solution due to gravity. The size of the particle as drawn and the probability density curve $P(h)$ for the center of mass height is approximately to scale for a 1 μm sphere with time measured in units of m/γ.

$=2Dt$. Although it is more likely that the particle moves downward, at short times there is a reasonable chance that thermal noise will cause the particle to be found slightly higher than where it started. Because the distance from the center of the Gaussian to the original position h_i is $(mg/\gamma)t$, the probability for a particle to be above its starting point at time t is $1/2 \, \mathrm{erfc}[((mg/\gamma)t)^2/4Dt]$, where $\mathrm{erfc}(x)$ is the complement of the error function.

These upward trajectories have been termed violations of the second law of thermodynamics on short time and small length scales[10] because the entropy change is negative for these trajectories.[11] The upward trajectories do not demonstrate a violation of the more relevant Thomson (Lord Kelvin) formulation of the second law which states that no repeatable (cyclic) process can do work on the environment with the sole change being a decrease in the temperature of the system. However, the upward trajectories can be exploited in a nonisotropic system to allow the random input of energy to drive directed motion by a Brownian motor mechanism.[12,13]

If we transpose h_i and h_j in the probability density to obtain $P(h_i,t|h_j,0) \equiv P(-h,t)$ in Eq. (2), we find the probability density for a particle to be at h_i at t, given that it started at h_j at $t=0$. The ratio $P(h,t)/P(-h,t)$ is

$$\frac{P(h,t)}{P(-h,t)} = e^{-mgh/\gamma D}. \qquad (4)$$

Remarkably, time has disappeared altogether in Eq. (4), and substituting Einstein's relation[14] $\gamma D = k_B T$, we regain the equilibrium barometric law, Eq. (1), which now relates the conditional probability to be at h_j at t given that it was at h_i at $t=0$ to the conditional probability to be at h_i at t given that it was at h_j at $t=0$. An analogous relation for an overdamped particle in an arbitrary potential was derived in a more general context in Ref. 15 using Onsager's thermodynamic action approach.[4]

Because the probability density function is normalized, $\int_{-\infty}^{+\infty} P(h,t)dh = \int_{-\infty}^{+\infty} P(-h,t)dh = 1$, we also have

$$\langle e^{mgh/k_B T}\rangle = \int_{-\infty}^{+\infty} e^{mgh/k_B T}P(h)dh = 1. \qquad (5)$$

The quantity mgh is the energy lost when a particle falls a distance h.

Relations analogous to Eqs. (4) and (5), known as generalized fluctuation-dissipation theorems, were derived from a very different perspective by Bochkov and Kozovlev.[16]

IV. MICROSCOPIC REVERSIBILITY AND DETAILED BALANCE

We can understand the origin of Eq. (4) in terms of the principle of microscopic reversibility[15]—the idea that at equilibrium every process is as likely as the microscopic reverse of that process. The equilibrium probability to observe a particle move from some position h_i at time 0 to a position h_j at time t by any trajectory is $c_{eq}(h_i)P(h_j,t|\cdots|h_i,0)$, which at equilibrium must equal the probability to observe the exact reverse process,

$$c_{eq}(h_i)P(h_j,t|\cdots|h_i,0) = c_{eq}(h_j)P^{\dagger}(h_i,t|\cdots|h_j,0). \qquad (6)$$

From Eq. (1), we thus have[15]

$$\frac{P(h_j,t|\cdots|h_i,0)}{P^{\dagger}(h_i,t|\cdots|h_j,0)} = e^{-mgh/k_B T}. \qquad (7)$$

for any path, where the $\dagger$ indicates the reverse trajectory. Although Eq. (7) was derived using knowledge of the behavior of the system at equilibrium, it is valid irrespective of how different the actual concentration distribution is from the equilibrium distribution. The conditional probabilities $P(h_i,t|\cdots|h_j,0)$ and $P^{\dagger}(h_j,t|\cdots|h_i,0)$ are defined independent of any reference to the concentrations themselves.[17]

The derivation of Eq. (7) is analogous to the use of detailed balance[18] (a corollary of microscopic reversibility) to derive that the ratio of rate constants for a chemical reaction is equal to the exponential of the free energy difference over the product of the gas constant and temperature, $\exp(\Delta G_0/RT)$. Consider a simple reaction

$$A \underset{k_r}{\overset{k_f}{\rightleftharpoons}} B, \qquad (8)$$

where k_f and k_r are the forward and reverse rate coefficients, respectively. Detailed balance dictates that $k_f c_{eq,A} = k_r c_{eq,B}$, where $c_{eq,A}$ and $c_{eq,B}$ are the equilibrium concentrations of species A and B, respectively. Because $c_{eq,B}/c_{eq,A} = \exp(\Delta G_0/RT)$, we also have $k_f/k_r = \exp(\Delta G_0/RT)$, a relation that holds irrespective of how different the actual concentration ratio $c_A(t)/c_B(t)$ is at any time from the equilibrium ratio $c_{eq,B}/c_{eq,A}$.

V. SINGLE MOLECULE EXPERIMENTS

Interest in the generalized fluctuation-dissipation theorem has recently been rekindled, particularly by the work of Evans,[19] Jarzynski,[20] and Crooks[21] who have made important progress by relating experimental observables, such as energy dissipation, and work done by an external force in single molecule experiments to thermodynamic parameters, such as the Gibbs or Helmholtz free energy. Hummer and Szabo[22] pointed out that the relations are implicit in the Feynman-Kac path integral theorem and demonstrated how the nonequilibrium work energy relations and fluctuation theorems can be used to interpret single molecule experiments. Several relations derivable from the generalized fluctuation-dissipation theorem have been tested experimentally by Bustamante and colleagues[23,24] who showed that a

relatively small number of single molecule experiments can be used to obtain very good estimates of the equilibrium free energy changes in the system.

It is often stated that many single molecule experiments are carried out "far from equilibrium." The 10–100 nN force typical for an AFM pulling experiment is enormous compared to the 10–20 pN force associated with even strong molecular motors[25] and the rate of change of the force–up to several hundred nN per second–seems very large. However, when we compare the rate of change of the force ($dF/dt \sim 100$ nN/s) with the ratio of the characteristic force[3] $F_{\text{char}} = \eta^2/\rho \sim 10^{-9}$ N to the characteristic relaxation time for a nanometer object in solution $t_{\text{char}} = \rho r^2/\eta \propto m/\gamma \sim 10^{-12}$ s ($F_{\text{char}}/t_{\text{char}} \sim 10^3$ N/s), we see that the system is very close to mechanical equilibrium at all times. The characteristic quantities are written in terms of the particle radius $r \sim 10^{-9}$ m, density $\rho \sim 10^3$ kg/m^3, and the viscosity of the solution $\eta \sim 10^{-3}$ kg/(m s). The closeness to mechanical equilibrium allows writing the microscopic reversibility conditions, Eqs. (6) and (7), to be written in terms of only the position of the particle. On time scales long compared to t_{char}, the time scale for thermal relaxation, it is implicit that the velocity distribution is the same everywhere and given by the Maxwell velocity distribution function. Although the system is in mechanical equilibrium, the system is far from thermodynamic equilibrium as it continues to move and dissipate energy to its environment. How can we describe such systems theoretically?

VI. LANGEVIN EQUATION FOR SINGLE MOLECULE STRETCHING

Onsager and Machlup[4] proposed a model for a thermodynamic nonequilibrium stochastic process based on the equation $R\dot{\alpha} = X + \epsilon$, where α is a generalized displacement, X is a generalized force, R is a transport coefficient, and ϵ is a Gaussian distributed random force. The key physical approximation is that the flux $\dot{\alpha}$ depends linearly on the force X that causes it. Typical macroscopic laws obeying such a relation are Ohm's law for electrical conduction, Fick's law for diffusion, and Fourier's law for heat conduction. Important for our considerations here, at a low Reynolds number[3] (which is the case for essentially all nanometer-scale motions of macromolecules in solution) the velocity (not the acceleration!) is proportional to the instantaneous local mechanical force[26] and hence can be described by an equation of the form given in Ref. 4.

Consider a macromolecule surrounded by water and tethered between a flat surface and the tip of an AFM (Fig. 2). The end-to-end extension of the molecule, h, depends on many intramolecular interactions which give rise to an internal potential[27] $U(h)$ as well as on an external, possibly time dependent force $g(t)F_{\text{ext}}$ applied using the AFM. Hence, the net force is $F = g(t)F_{\text{ext}} - U'(h)$. Following Onsager, we describe stretching of the macromolecule by a Langevin equation:[28–30]

$$\gamma\dot{h} = F + \sqrt{2\gamma k_B T}\xi(t). \tag{9}$$

The effect of the aqueous solution is modeled in terms of two forces, a thermal noise term $\sqrt{2\gamma k_B T}\xi(t)$, which fluctuates rapidly compared to all other time scales in the system, and a viscous drag force $\gamma\dot{h}$, which slows the motion induced by
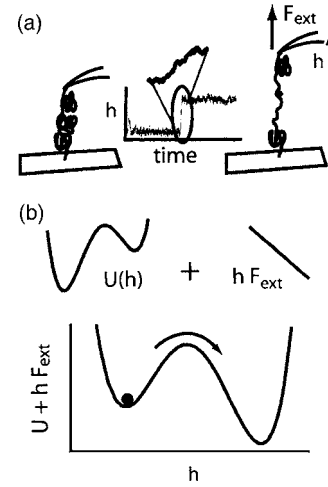

Fig. 2. (a). In a typical single molecule stretching experiment a protein is attached at one end to the cantilever of an AFM and at the other to a substrate on a translatable stage. A force is applied through the cantilever tip and maintained constant via feedback. After a waiting period, the molecule undergoes an unfolding transition thereby relieving its internal tension. A plot of the extension h versus time, obtained by simulation of the Langevin equation (9) [using Eq. (A1)] with a bistable potential is shown, with the trajectory near the transition illustrated in detail in the exploded view. The external force was taken to be constant in time [$g(t)=1$]. (b). Bistable potential resulting from the combination of the potential of mean force arising from internal chemical interactions and the external force.

the fluctuation term. The amplitude $\sqrt{2\gamma k_B T}$ reflects the fluctuation-dissipation relation between the random collisions with solvent molecules that accelerate the particle and the viscous drag of the fluid that counteracts this acceleration. The time dependence of the random force is modeled as a Gaussian noise with zero mean and standard deviation $dt^{-1/2}$ which in the limit $dt \to \infty$ becomes white noise[31] with the statistical properties $\langle\xi(t)\rangle = 0$ and $\langle\xi(t)\xi(t')\rangle = \delta(t-t')$. It can be shown that the probability density function $P(h,t)$ for the stochastic process defined by Eq. (9) satisfies the partial differential equation (3) with mg replaced by $-F$. In this slightly generalized context, Eq. (3) is known as the forward Fokker-Planck equation.[32]

The net mechanical force $F = g(t)F_{\text{ext}} - U'(h)$ is the combination of the gradient of the internal potential, $U'(h)$ and the external force $g(t)F_{\text{ext}}$. The work (force × displacement) done by the external forcing over the time t,

$$w_{\text{ext}}[h(t)] = \int_0^t ds[g(s)F_{\text{ext}} \times \dot{h}], \tag{10}$$

depends on the velocity $\dot{h}$, and the reversible work done on the system by moving the particle in the internal potential,

$$\Delta U = \int_0^t ds[U'(h) \times \dot{h}] = U(h(t)) - U(h(0)), \tag{11}$$

depends only on the endpoints. The difference, $w_{\text{dis}}[h(t)] = w_{\text{ext}}[h(t)] - \Delta U$, is the dissipated energy.

VII. ONSAGER-MACHLUP THERMODYNAMIC ACTION APPROACH

Given the initial condition $h(0)$, the specification of $\xi(t)$ in Eq. (9) defines a unique trajectory for $h(t)$. For discrete time $t_k = k\Delta t$, $k=0,1,\dots,n$ we have

$$P(\xi_k) \propto e^{-\xi_k^2 \Delta t/2}. \tag{12}$$

Because the noise is "delta correlated" $\langle \xi_k \xi_l \rangle = \Delta t^{-1} \delta_{k,l}$, the probabilities of the values of ξ in any sequence are independent, and we can write[33]

$$P[(\xi_n,t|\dots|\xi_0,0)] \propto \prod_{k=0}^{n} e^{-\xi_k^2 \Delta t/2} = e^{\sum_{k=0}^{n} -\xi_k^2 \Delta t/2}. \tag{13}$$

In the limit $\Delta t \to 0$ the sum becomes an integral, and we can write the probability of a trajectory as[15]

$$P[\xi(t)] \propto e^{-\int_0^t ds\, \xi^2(s)/2}. \tag{14}$$

From Eq. (9), $\xi(t) = (\dot{h} - F/\gamma)/\sqrt{2D}$, so we can further write

$$P[\xi(t)] \propto e^{-S/D}, \tag{15}$$

where

$$\frac{S}{D} = \frac{1}{4D} \int_0^t ds[\dot{h} - F/\gamma]^2$$

$$= \frac{\Delta U - w_{\text{ext}}[\xi(t)]}{2\gamma D} + \frac{1}{4D} \int_0^t ds(\dot{h}^2 + F^2/\gamma^2) \tag{16}$$

is the thermodynamic action divided by the diffusion coefficient.[30] To find the most probable path leading from $(h_i,0)$ to (h_j,t), we maximize the probability $P[\xi(t)]$ subject to the endpoints, which requires that the action Eq. (16) be minimized.[34]

By taking the ratio of the probability for the forward and reverse path, we immediately obtain the generalization of Eq. (7). In the reverse path $\dot{h} \to -\dot{h}$, so $w_{\text{ext}}[\xi^\dagger(t)] = -w_{\text{ext}}[\xi(t)]$ and $\Delta U^\dagger = -\Delta U$, but the argument of the integral in the second line in Eq. (16) is unchanged and we have[15]

$$\frac{P[\xi(t)]}{P[\xi^\dagger(t)]} = e^{(w_{\text{ext}}[\xi(t)] - \Delta U)/k_B T}, \tag{17}$$

where we used $k_B T = \gamma D$. The work probability density function is given by

$$P(W_{\text{dis}}) = \int_{\xi(t)} d\xi'(t)\, \delta(W_{\text{dis}} - w_{\text{dis}}[\xi'(t)]) P[\xi'(t)]. \tag{18}$$

For every trajectory with dissipated work W_{dis}, there is a corresponding reverse trajectory with dissipated work $-W_{\text{dis}}$. The ratio of the probabilities for forward and reverse trajectories is given by Eq. (17), so the ratio of the work probabilities is

$$\frac{P(W_{\text{dis}})}{P(-W_{\text{dis}})} = e^{W_{\text{dis}}/k_B T}. \tag{19}$$

Because $\int_{-\infty}^{\infty} P(W_{\text{dis}}) dW_{\text{dis}} = \int_{-\infty}^{\infty} P(-W_{\text{dis}}) dW_{\text{dis}} = 1$, we also have

$$\langle e^{-W_{\text{dis}}/k_B T} \rangle = \int_{-\infty}^{\infty} dW_{\text{dis}} e^{-W_{\text{dis}}/k_B T} P(W_{\text{dis}})$$

$$= \int_{-\infty}^{\infty} dW_{\text{dis}} P(-W_{\text{dis}}) = 1. \tag{20}$$

The second law of thermodynamics states that the average work done in any process is greater than (or in the reversible case equal to) the energy stored, $\langle W_{\text{ext}} - \Delta U \rangle \geqslant 0$. The second law is, by Jensen's inequality,[35] consistent with, but does not require, Eq. (20).

Equations (19) and (20), which follow immediately from Eq. (17), agree with the generalized fluctuation dissipation theorem of Bochkov and Kuzovlev,[16] the derivation of Eq. (20) as a nonequilibrium work relation by Jarzynski,[20] and the derivation of Eq. (19), the entropy production fluctuation theorem, by Crooks.[21] We have derived these relations from the Onsager-Machlup thermodynamic action approach[4] based on the approximation that acceleration $(\ddot{h})$ is negligible and the assumption that the underlying noise $\xi(t)$ is Gaussian distributed in the stochastic description of the system by the Langevin equation (9). This approximation and assumption are extremely well founded for single molecules in solution. The former is assured by the small size of the molecules and by the viscosity of the solution. The latter is assured by the central limit theorem, because the macroscopic motion of a molecule is the result of an enormous number of individual molecular collisions.[14]

Equations (19) and (20) are remarkable in their simplicity and in the fact that they do not depend on time. Suppose that we start a system in any configuration, with an arbitrary external force having any time dependence, and allow the system to evolve for an arbitrary time. Equation (19) says that the ratio of the probability of receiving a certain sequence of "kicks" from the environment in which an amount of energy W_{dis} is dissipated to the probability to receive a sequence of kicks in which the energy dissipated is $-W_{\text{dis}}$ equals $e^{W_{\text{dis}}/k_B T}$. Further, Eq. (20) says that if we do the experiment many times (but not necessarily starting always in the same configuration) and take the exponential average, we would find $\langle e^{-W_{\text{dis}}/k_B T} \rangle = 1$. These statements are valid arbitrarily far from thermodynamic equilibrium, but were derived here based on approximating the system as being in mechanical equilibrium so that the inertial term $m\ddot{h}$ that would otherwise appear in Eq. (9) can be ignored. It remains an open question as to whether Onsager's extension of his approach to systems with kinetic energy[36] can be used to generalize the applicability of Eqs. (19) and (20) to include systems where $m\ddot{h}$ is not negligible.

Note that we have only considered situations, where the process of interest (for example, motion of colloidal particles and extension of a polymer) is away from thermodynamic equilibrium, but not the medium in which the process occurs. It is implicitly assumed that the medium is in equilibrium and hence the diffusion and viscosity coefficients and the temperature are well defined. For this reason, the derivation of Eq. (20) from the Langevin equation (9) does not directly speak to the criticism of Cohen and Mauzerall[37] who argue that Eq. (20) is not an identity in systems where temperature is ill defined.

VIII. CONCLUSIONS

In nanoscale physics, it is essential to remember that "equilibrium" does not have an unambiguous meaning. The nonequilibrium aspect of a typical experimental system shows up as a mean energy dissipation rate that is nonzero. The mechanical equilibrium aspect shows up in the absence of acceleration.[3]

When Onsager-Machlup theory is mentioned, the almost uniform response by scientists is that the Onsager approach is applicable only near equilibrium in the linear response regime. Although these restrictions are technically correct, they are also very misleading. The equilibrium required for application to single molecule studies is that the system be near to *mechanical equilibrium*. For macromolecules in solution, it is almost impossible to achieve a situation where this condition is not the case. Further, linear response requires that the velocity depend linearly on the net force that causes it, not necessarily on the externally applied force.[26] Also, although it is required that the underlying noise process $[\xi(t)]$ in Eq. (9) be Gaussian, it is not required (nor in general expected) that functions of the noise such as the position and work be themselves Gaussian.

Mechanical equilibrium allows a simple description of the distribution of the fluctuations of quantities about their mean values in thermodynamically far from equilibrium situations. The closeness to mechanical equilibrium is ultimately the explanation for the "unreasonable" effectiveness of equilibrium theory for interpreting single molecule experiments that are far from thermodynamic equilibrium.

Time independent relations that hold for nonequilibrium and even nonstationary processes offer great possibility for the rapid determination of thermodynamic parameters from single molecule experiments. This paper is intended to provide a background for understanding these equilibriumlike relations and to shine light on their historical origins.

APPENDIX A: SUGGESTED PROBLEMS—DISCRETIZING THE LANGEVIN EQUATION

By discretizing Eq. (9) at times $t_k = k\Delta t$ for $k = 0, 1, 2, \ldots, n$, we obtain the update equation[31] suitable for numerical evaluation

$$h_{k+1} = h_k + \gamma^{-1}F_k\Delta t + \sqrt{2D\Delta t}N_k, \tag{A1}$$

where[15] $F_k = g(t_k)F_{\text{ext}} - 1/2[U'(h_{k+1}) + U'(h_k)]$ and the $N_k = N(0,1)$ are independent Gaussian random variables of zero mean $\mu = 0$, unit standard deviation $\sigma = 1$, and take on values n_k according to the density function

$$P(n_k) = \frac{1}{\sqrt{2\pi}}e^{-n_k^2/2}. \tag{A2}$$

We used the relation $N(\mu, \sigma^2) = \mu + \sigma N(0,1)$ to convert to a unit normal Gaussian random variable, where μ is the mean, and σ is the standard deviation of the distribution. To carry out a simulation, we substitute a sample value n_k for the Gaussian random variable N_k, carry out the indicated arithmetic in Eq. (A1), and proceed to the next iteration, thus generating a sequence of positions and times $(h_k, k\Delta t)$. From the sequence, any function of position and time can be calculated. We choose Δt long compared to the velocity relaxation time t_{char}, so that the noise n_k reflects an average over many individual molecular collision, but short enough that the maximum potential energy change due to the distance traveled in Δt, $U'(h)\Delta h/\Delta t$, is small compared to the thermal energy k_BT. An example of the simulated motion in the combined potential is shown in Fig. 2(a), where the trajectory between the two wells is shown in detail.

Equation (A1) provides a computationally simple way to simulate the motion of systems for which thermal noise is significant but for which acceleration is negligible. The discussion and manipulation of the resulting data sets (series of positions versus time) provides an excellent way for students to gain practice and insight into the analysis of the types of data typically obtained in single molecule pulling experiments. I have used the following three problems in teaching molecular biophysics.

Problem 1. Demonstrate explicitly the validity of Eq. (17) for a Langevin simulation using Eq. (10) for the harmonic potential, $U = kh^2$. Discuss the difference between the probability for a trajectory between two values h_i and h_j from 0 to t (the relative frequency with which such a trajectory is observed) and the conditional probability to observe a trajectory ending at $h_j(h_i)$ at t given that the particle started at $h_i(h_j)$ at 0. Discuss the tradeoffs involved in the decision as to whether to use large time steps Δt and large bin sizes dh or small time steps and small bin sizes.

Problem 2. Calculate the work and dissipation for several well-to-well transitions for a quartic potential such as that shown in Fig. 2. Plot the work probability densities analogous to those used in Ref. 23 and estimate the number of trajectories necessary to obtain an adequate statistical weight near the tails of the distributions (regions far from the maximum) to draw conclusions about the validity of Eq. (19) and Eq. (20).

Problem 3. In regions that are neither near a maximum nor a minimum of the potential energy the motion of the particle is tightly distributed around a most probable trajectory[4] defined by[15] $\dot{h} = \pm F/\gamma$. Calculate the most probable trajectory for transition between wells in a bistable potential and compare to several simulated transitions using Eq. (A1). Discuss how the most probable trajectory obtained by averaging over many stochastic simulations (or single molecule experiments) could be used to directly obtain the underlying potential energy profile.

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[11] Although the colloid described here is a single particle, there are many internal degrees of freedom. The state of maximum entropy is one in which the energy in the system is distributed equally over all degrees of freedom. When a heavier than water particle moves up in water, the energy distribution becomes less homogeneous, a situation that can arguably be described as one in which the entropy of the system has decreased. A very interesting question is how large a system must be in order for the concept of entropy to be meaningful.

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[17] It has become increasingly common to see articles with the terms "nonequilibrium trajectories" or "nonequilibrium paths" appearing even in the titles and abstracts. Strictly speaking, these terms are meaningless. A path is simply a sequence of positions in space, and a trajectory is a sequence of positions and times in spacetime. Although we can reasonably ask for the probability that a given trajectory was drawn from some particular equilibrium ensemble of trajectories, it makes no more sense to refer to a "nonequilibrium trajectory" than it would to talk about a "nonequilibrium velocity" for a molecule in a system at a given temperature.

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[34] In changing variables from $\xi(t)$ to $h(t)$ the probability densities must satisfy $\int P[h(t)]dh = \int P[\xi(t)]d\xi$ for any set of trajectories. Hence $P[h(t)] = \|d\xi/dh\|P[\xi(t)]$, where $\|d\xi/dh\| \equiv J[h(t)]$ is the Jacobian matrix that compensates for the nonlinear stretching and shrinking involved in transforming from trajectories in noise space $\xi(t)$ to trajectories in the space $h(t)$. An explicit expression for the Jacobian has been derived, but because it does not enter into calculations of the ratios of probability densities, or in calculations for path probabilities to first order in noise strength, we will not consider it further here.

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