Nonequilibrium work relations: foundations and applications

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Abstract. When a macroscopic system in contact with a heat reservoir is driven away from equilibrium,
the second law of thermodynamics places a strict bound on the amount of work performed on the
system. With a microscopic system the situation is more subtle, as thermal fluctuations give rise to a
statistical distribution of work values. In recent years it has been realized that such distributions encode
surprisingly more information than one might expect from traditional thermodynamic arguments. I will
discuss a number of exact results that relate equilibrium properties of the system, in particular free
energy differences, to the fluctuations in the work performed during such a nonequilibrium process. I
will describe the theoretical foundations of these relations, connections with irreversibility and the second
law of thermodynamics, and potential experimental and computational applications.

PACS. 05.70.Ln Nonequilibrium and irreversible thermodynamics – 05.40.-a Fluctuation phenomena,
random processes, noise, and Brownian motion – 82.37.Rs Single molecule manipulation of proteins and
other biological molecules

1 Introduction

The focus of this contribution is a set of results pertaining to systems that are driven away from an initial state of thermal equilibrium. In principle these results are valid quite generally, but in practice they are relevant mainly for microscopic systems. Indeed, the topic that I will discuss represents one small corner of a considerably broader, active field of research, which might be called the *thermodynamics of small systems* [1]. The question at the heart of this field is how (and to what extent, and in what forms) the laws of thermodynamics – originally formulated to describe the behavior of macroscopic systems such as steam engines – apply to microscopic systems such as biomolecular machines. As an example, consider the RNA polymerase, which attaches itself to a double strand of DNA, marches along that strand, and copies a piece of the genetic code onto a single strand of RNA, occasionally backtracking to correct a copying error. While the RNA polymerase and the steam engine are separated by about eight orders of magnitude in length scale, they both function by using some form of free energy to carry out a specific task, whether that task involves pumping water out of a mine shaft, or copying a piece of genetic code. How do the laws developed by Sadi Carnot and his successors apply to microscopic systems such as the RNA polymerase?

While the preceding comments hopefully convey a sense of the broader context in which to understand this
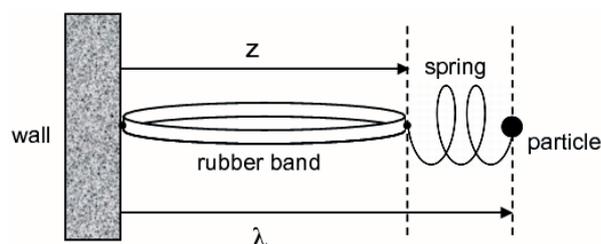
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Fig. 1. Stretching a rubber band.

contribution, I will focus on a very specific topic, which involves the relationship between work and free energy in nonequilibrium thermodynamic processes. With that in mind, let me begin with a very simple, pedagogical example of such a process.

2 Stretching a rubber band

Imagine an ordinary rubber band, attached at one end to a fixed wall, and at the other to an ideal spring, as depicted in Figure 1. Let z denote the length of the rubber band, and λ the distance from the fixed wall to a particle attached to the far end of the spring. We will view λ as a degree of freedom that we control directly, by manipulating the position of the particle. We recognize here the basic elements of a textbook discussion of thermodynamic processes: a *system of interest*, the rubber band; a *thermal*
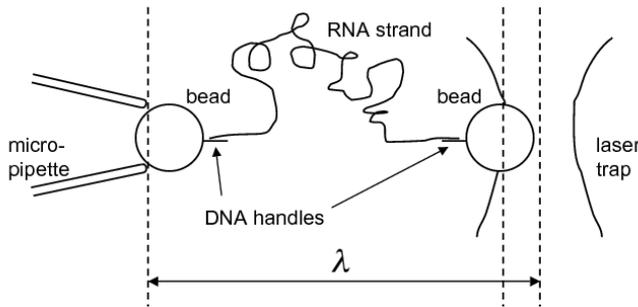
Fig. 2. Stretching a single molecule (*not to scale*).

environment, the surrounding air; and a *work parameter*, λ, used to act on the system of interest.

With these elements let us imagine that we subject the system to a nonequilibrium process, as follows. The system begins in a state of thermal equilibrium with its surroundings, with the parameter at some initial value, $\lambda = A$. Then we stretch the rubber band by varying the work parameter, from A to a final value $\lambda = B > A$. Let us imagine that this is done rather rapidly and violently, so that the rubber band heats up – as rubber bands do, when they are stretched rapidly – and thus is driven away from equilibrium with the surrounding air. During this step we perform *work*, W, on the system. Finally, imagine that at the end we hold the work parameter fixed at $\lambda = B$, allowing the rubber band to cool off and once again equilibrate with its environment. We thus have an irreversible process during which external work is performed in driving the system from one equilibrium state (A) to another (B).

For such a process the second law of thermodynamics asserts that the work must be no less than the free energy difference, ΔF, between the two equilibrium states:

$$W \geq \Delta F = F_B - F_A, \tag{1}$$

where the equality $(W = \Delta F)$ holds if we perform the process reversibly. Formally, equation (1) follows from the Clausius inequality, generally written in terms of heat and entropy $(\int dQ/T \leq \Delta S)$ rather than work and free energy. We can also understand equation (1) phenomenologically: when a rubber band is stretched rapidly, it heats up, and when it heats up its tension increases. Consequently, more work is required to stretch a rubber band irreversibly, than reversibly.

3 Stretching a single molecule

Now consider what happens during a single-molecule pulling experiment, depicted schematically in Figure 2. In this microscopic analogue of a stretched rubber band, DNA handles are used to "glue" a strand of RNA to two microspheres (polystyrene beads), one held in place by a micropipette, the other confined by an optical trap. The entire system is immersed in aqueous solution at room temperature and pressure, and we manipulate the distance from the end of the pipette to the center of the optical trap, λ, by varying the position of the trap. In this
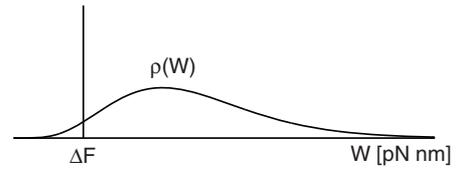

Fig. 3. Distribution of work values for a microscopic system subject to an irreversible process.

example the system of interest is the RNA strand tethered to the beads, the thermal environment is the surrounding water, and λ once more denotes the work parameter. The optical trap plays the role of the spring in Section 2, creating an approximately harmonic potential for the bead.

We again imagine an irreversible process: first the system is prepared in equilibrium at $\lambda = A$; then we perform work (W) on the RNA strand as we stretch it by varying λ from A to B according to some pre-determined schedule, or *protocol*; and finally the system is allowed to once again reach thermal equilibrium, with the pipette-to-trap distance held fixed at $\lambda = B$.

Suppose we perform this experiment repeatedly, always preparing the system in the same equilibrium state, and always following the same protocol when manipulating the work parameter from A to B. The work we perform nevertheless differs measurably from one realization to the next, due to the inevitable randomness of atomic and molecular motions. In other words, thermal fluctuations of the RNA and surrounding water molecules give rise to statistical fluctuations in the work required to stretch the biomolecule. In this case the Clausius inequality must be interpreted *statistically*. This is illustrated in Figure 3, where $\rho(W)$ depicts the distribution of work values observed over many realizations of such a process. The average work exceeds the free energy difference between the initial and final states of the system,

$$\langle W \rangle \geq \Delta F, \tag{2}$$

but there is a substantial spread of work values around this average. As we repeatedly perform this experiment, we might even observe occasional realizations for which W falls below ΔF, as depicted by the far left tail of $\rho(W)$ in Figure 3. These "violations of the second law" [2] are fortuitous events, during which random thermal fluctuations interfere constructively (so to speak) in a manner that facilitates the stretching process.

4 Nonequilibrium work relations

The observations of the previous section represent a reasonable, statistical extrapolation of the second law of thermodynamics to the realm of microscopic systems. It turns out, however, that one can make considerably stronger statements regarding the fluctuations in work.

One such statement is the following *nonequilibrium work relation* [3,4]:

$$\left\langle e^{-\beta W} \right\rangle = e^{-\beta \Delta F}. \tag{3}$$

Here as in equation (2), angular brackets denote an average over a statistical ensemble of realizations of a given thermodynamic process, equivalently an integral over the observed work distribution, $\rho(W)$. The factor $\beta = 1/k_B T$ is the inverse temperature of the surrounding thermal environment, and can also be viewed as the initial inverse temperature of the system itself, since the system is presumed to be prepared in equilibrium with its surroundings. (However, it would be incorrect to view β^{-1} as the temperature of the system *during the process*; indeed, the system might not even have a well-defined temperature as it is driven away from equilibrium.)

Equation (3) places a rather strong and unexpected constraint on the distribution of work values, $\rho(W)$, which (unlike Eq. (2)) is not naturally suggested by macroscopic experience. Two features make this result particularly interesting (and occasionally controversial [5–8]). First, it remains valid even if the system is driven far from thermal equilibrium. Thus it goes beyond the near-equilibrium predictions of linear response theory. Second, it asserts that we can determine equilibrium properties of a system of interest – specifically, free energy differences – by observing how the system responds when driven away from equilibrium. This feature renders equation (3) potentially useful in both experimental and computational contexts, as I will discuss in Section 8 below.

5 Forward and reverse processes, Crooks's fluctuation theorem

Equation (3) pertains to a thermodynamic process during which a work parameter λ is manipulated from A to B. A closely related prediction applies to a comparison of two process: the *forward* process just described; and a counterpart *reverse* process, during which λ is varied from B back to A, following a protocol that is the time-reversal of the one used during the forward process (Eq. (21)).

Let us first consider such a comparison in the macroscopic setting. As in Section 2, we imagine that we stretch the rubber band by moving the right end of the spring from A to B, say at some constant speed u. Then, after the rubber band has re-equilibrated with the surrounding air, we move the particle back in the opposite direction, again at speed u, from B to A. Let W_F and W_R denote the work performed during these two steps. The subscripts, for "forward" and "reverse", indicate that we treat these steps as distinct processes. However, we can equally well view the combination of the two, in succession, as a single thermodynamic cycle, $\lambda : A \rightarrow B \rightarrow A$. The work values $W_{F,R}$ must then be consistent with the Kelvin-Planck statement of the second law of thermodynamics [9], which forbids the systematic conversion of heat to work, when the heat is drawn during a cyclic process from a single thermal reservoir. In our setting this implies

$$W_F + W_R > 0. \qquad (4)$$

The work we perform to stretch the rubber band during the first half-cycle (W_F) must exceed the work that we
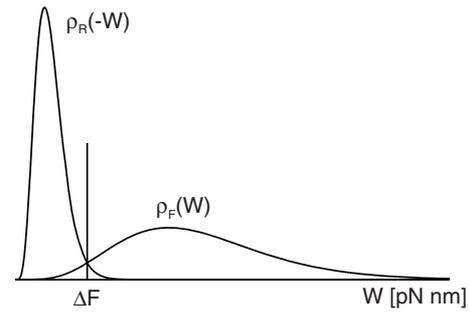
Fig. 4. Work distributions for the forward and reverse processes. Equation (5) is illustrated by the fact that the mean of $\rho_F(W)$ is found to the right of the mean of $\rho_R(-W)$.

recover in allowing the rubber band to contract during the second half-cycle ($-W_R$). This is essentially a "no free lunch" theorem. It rules out a perpetual motion machine of the second kind, by which energy would be harvested from the thermal motions in a roomful of air simply by repeatedly stretching and contracting a rubber band.

In the microscopic, single-molecule setting of Figure 2, imagine that we repeatedly move the laser trap back and forth between two positions A and B, measuring the work performed during each half-cycle, and allowing the strand of RNA to re-equilibrate with the surrounding water each time A or B is reached. Letting $\rho_F(W)$ denote the distribution of work values observed over many repetitions of the stretching step ($A \rightarrow B$), and defining $\rho_R(W)$ similarly for the contraction step ($B \rightarrow A$), the analogue of equation (4) is the statistical statement,

$$\langle W \rangle_F + \langle W \rangle_R > 0, \qquad (5)$$

with angular brackets denoting averages over the distributions ρ_F and ρ_R; see Figure 4. This result states that there is no free lunch *on average*: we might occasionally observe a cycle during which more work is recovered when the molecule contracts than was performed to stretch it in the first place, but such events are the exception rather than the rule.

While equation (5) is a statement about the *means* of the work distributions, the fluctuations around these means are interesting in their own right. Crooks has shown that ρ_F and ρ_R satisfy the symmetry relation [10,11],

$$\frac{\rho_F(+W)}{\rho_R(-W)} = e^{\beta(W - \Delta F)}. \qquad (6)$$

This result is closely related to various *fluctuation theorems* that have been derived for entropy production in out-of-equilibrium systems (see e.g. Refs. [12–21], and references therein), in particular to the *transient fluctuation theorem* of Evans and Searles [13,18].

Since equation (6) tells us that $\rho_F(W)$ is obtained by multiplying $\rho_R(-W)$ by a monotonically increasing function, of W, it immediately follows that the mean of $\rho_F(W)$ is located to the right of the mean of $\rho_R(-W)$, which is precisely the content of equation (5). Moreover, equation (6) implies that $\rho_F(W)$ and $\rho_R(-W)$ intersect at $W = \Delta F$ [22], as depicted in Figure 4.

Finally, by multiplying both sides of equation (6) by $\rho_R(-W)\exp(-\beta W)$, then integrating over all values of W, we obtain equation (3).

As was the case with equation (3), (6) remains valid even if the system is driven far from equilibrium during the forward and reverse processes.

6 Simple derivations

Over the past decade or so, a number of derivations of these and closely related results have appeared in the literature, see for instance references [3,4,10,11,23–38]. Moreover, this work has brought new attention to a set of results obtained nearly three decades ago by Bochkov and Kuzovlev [39–42]; see references [43–45] for discussions of the relation between these two sets of predictions. Here I will discuss three derivations of nonequilibrium work relations. While my focus will be limited to systems described using the methods of classical statistical mechanics, there has been considerable interest in extending these results to the quantum regime, as found in references [46–56].

6.1 Hamiltonian evolution

First, consider the special case in which the system is thermodynamically isolated – that is, not in contact with a thermal reservoir – as the work parameter is varied from A to B. Specifically, imagine a hypothetical single-molecule pulling experiment that is prepared by allowing the trapped strand of RNA to equilibrate with a gas-phase environment (e.g. air at room temperature and pressure), rather than in aqueous surroundings. The gas is then evacuated, leaving only the molecule attached to the beads, along with the manipulation apparatus, i.e. the optical trap used to grab one of the polystyrene beads, and some sort of anchor to hold the other bead fixed. This is not a physically realistic situation, but the purpose here is simply to illustrate how a result like equation (3) might be derived from first principles, while avoiding some of the technical details that arise when properly accounting for the degrees of freedom of the thermal environment. Once the system has been thus prepared in equilibrium, and the thermal reservoir removed, we stretch the molecule in vacuum by moving the optical trap from A to B. The process is then repeated many times, always first preparing the system in equilibrium with thermal surroundings, which are then removed.

Because the system is thermodynamically isolated as λ is switched from A to B, we use Hamilton's equations to describe its microscopic evolution. Specifically, let $x = (\mathbf{q}, \mathbf{p})$ denote a *microstate* of the system, that is a single point in its many-dimensional phase space, which includes all the relevant coordinates needed to specify the microscopic configuration of the system ($\mathbf{q}$) as well as the momenta associated with these coordinates ($\mathbf{p}$).

Next, let $H(x; \lambda)$ be the Hamiltonian that: (i) gives the internal energy of the system as a function of its microstate x; and (ii) generates the microscopic evolution,

$$\dot{\mathbf{q}} = \frac{\partial H}{\partial \mathbf{p}}, \quad \dot{\mathbf{p}} = -\frac{\partial H}{\partial \mathbf{q}}. \tag{7}$$

Note that the position of the optical trap, λ, enters as a parameter of the Hamiltonian. When this system is equilibrated with a thermal environment, at fixed λ, its microstate can be viewed as a random variable sampled from the Boltzmann-Gibbs distribution

$$p_\lambda^{\mathrm{eq}}(x) = \frac{1}{Z_\lambda} \mathrm{e}^{-\beta H(x;\lambda)}. \tag{8}$$

The partition function and free energy associated with this equilibrium state are given by expressions familiar from classical statistical mechanics:

$$Z_\lambda = \int \mathrm{d}x\, \mathrm{e}^{-\beta H}, \quad F_\lambda = -\beta^{-1} \ln Z_\lambda. \tag{9}$$

(Strictly speaking, the partition function includes a factor that makes it dimensionless. Since this factor would in any case drop out later in our analysis, because we are ultimately interested in free energy *differences*, we omit it from the start.)

During a given realization of the process, after being prepared in equilibrium the system evolves in time (Eq. (7)) as λ is switched from $\lambda_0 = A$ to $\lambda_\tau = B$. Here the notation λ_t, $0 \leq t \leq \tau$, denotes the externally imposed schedule, or *protocol*, for varying the work parameter; and x_t denotes the phase space evolution of the system. Because the system is thermally isolated from $t = 0$ to $t = \tau$, the work performed is simply the net change in its internal energy: $W = H(x_\tau; B) - H(x_0; A)$. This is just the first law of thermodynamics, taking into account that there is no heat transfer ($Q = 0$) because the system is isolated.

Let us now evaluate the average of $\mathrm{e}^{-\beta W}$, over an ensemble of realizations of this process. Since Hamiltonian evolution is deterministic, we can express the work W as a function of the initial conditions:

$$W(x_0) = H\left(x_\tau(x_0); B\right) - H(x_0; A). \tag{10}$$

Here $x_\tau(x_0)$ denotes the final conditions in phase space, for the trajectory launched from initial conditions x_0. The left side of equation (3) is then an average of $\exp[-\beta W(x_0)]$, over initial conditions sampled from the equilibrium distribution at $\lambda = A$:

$$\left\langle \mathrm{e}^{-\beta W} \right\rangle = \int \mathrm{d}x_0\, p_A^{\mathrm{eq}}(x_0)\, \mathrm{e}^{-\beta W(x_0)} \tag{11}$$

$$= \frac{1}{Z_A} \int \mathrm{d}x_0\, \mathrm{e}^{-\beta H(x_\tau(x_0);B)} \tag{12}$$

$$= \frac{1}{Z_A} \int \mathrm{d}x_\tau \left| \frac{\partial x_\tau}{\partial x_0} \right|^{-1} \mathrm{e}^{-\beta H(x_\tau;B)}. \tag{13}$$

We get from the first line to the second by substitution of equations (8) and (10); then to the third line by changing the variables of integration from x_0 to $x_\tau(x_0)$. Such a

change of variables is permitted since initial conditions x_0 and final conditions x_τ are in one-to-one correspondence under Hamiltonian evolution. The additional factor in the last line is the Jacobian associated with this change of variables: if we evolve an infinitesimal cell in phase space containing x_0, under Hamilton's equations, to a cell containing $x_\tau(x_0)$, then $|\partial x_\tau / \partial x_0|$ is the ratio of the phase space volumes of these cells. By Liouville's theorem this ratio is exactly unity, hence

$$\left\langle e^{-\beta W} \right\rangle = \frac{1}{Z_A} \int dx_\tau \, e^{-\beta H(x_\tau; B)} = \frac{Z_B}{Z_A} = e^{-\beta \Delta F}. \quad (14)$$

It must be stressed that the derivation just presented applies under the restrictive conditions of an *isolated* system. It was this assumption that allowed us to write W as the change in the system's internal energy; to use Hamilton's equations to describe its microscopic evolution; and finally to invoke conservation of phase space volume (Liouville's theorem). However, this Hamiltonian approach can be generalized [28] to encompass the more interesting situation in which the system continues to exchange energy (heat) with the reservoir – e.g. the surrounding water – as the work parameter is varied from A to B. In the generalized derivation, Hamilton's equations apply to motion in the *full* phase space describing both the system and the thermal reservoir. Apart from technical complications involving the free-energetic cost of solvating the RNA strand in the surrounding water, the steps used to obtain equation (3) are essentially the same as those outlined above.

6.2 Stochastic evolution

Now let us consider a different approach to modeling the microscopic evolution of a system driven away from thermal equilibrium. As before we will be interested in the evolution of the system over a time interval $0 \le t \le \tau$ as the work parameter λ is varied according to some predetermined protocol. However, following Crooks [10], now we will describe the microscopic history of the system as a sequence $x_0, x_1, \cdots x_N$, representing the points in phase space visited at times $t_0, t_1, \cdots t_N$, where $t_n = n\tau/N$. Moreover, let us assume that this evolution is a *Markov process*: given the microstate x_n at time t_n, the subsequent microstate x_{n+1} is sampled randomly from a transition probability distribution P that depends on x_n, but not on the points in phase space visited prior to time t_n. Physically, the randomness arises from the interactions with the thermal environment. We motivate the Markov assumption by assuming that this randomness is uncorrelated over time intervals longer than $\delta t = \tau/N$.

The transition probability to the next microstate x_{n+1} depends not only on the current microstate x_n, but also on the current value of the work parameter, λ_n. We will use the notation $P(x_n \to x_{n+1}; \lambda_n)$ to reflect this dependence. Moreover, we will assume that this transition probability obeys *detailed balance* [57]:

$$\frac{P(x \to x'; \lambda)}{P(x \leftarrow x'; \lambda)} = \frac{e^{-\beta H(x'; \lambda)}}{e^{-\beta H(x; \lambda)}}, \quad (15)$$

for any x, x', λ, where the arrows denote the sense of the transition.

When this system evolves as λ is varied in discrete steps from A to B, as during the forward process, the evolution during one time step proceeds by a sequence

$$(x_n, \lambda_n) \to (x_n, \lambda_{n+1}) \to (x_{n+1}, \lambda_{n+1}) \quad (16)$$

representing an update in the value of the work parameter, followed by a random step taken by the system. A trajectory from $t_0 = 0$ to $t_N = \tau$ is then generated by first sampling x_0 from the initial distribution p_A^{eq}, and then repeating the above sequence (Eq. (16)) in time increments δt. For the reverse process $(A \leftarrow B)$ the situation is much the same, except initial conditions are sampled from p_B^{eq}, and we assume that during a given time step the system first takes a random step, and then the work parameter is updated: $(x_{n+1}, \lambda_{n+1}) \leftarrow (x_{n+1}, \lambda_n) \leftarrow (x_n, \lambda_n)$.

The net change in the internal energy of the system, $\Delta E = H(x_N, \lambda_N) - H(x_0, \lambda_0)$, can be written as a sum of two contributions: the changes in energy due to increments in the work parameter,

$$W = \sum_{n=0}^{N-1} \left[H(x_n; \lambda_{n+1}) - H(x_n; \lambda_n) \right], \quad (17)$$

and those due to the transitions from one point in phase space to the next,

$$Q = \sum_{n=0}^{N-1} \left[H(x_{n+1}; \lambda_{n+1}) - H(x_n; \lambda_{n+1}) \right]. \quad (18)$$

As suggested by the notation, and as argued by Crooks [10], these two contributions are naturally interpreted as the *work*, W, performed on the system, and the *heat*, Q, that it absorbs from its surroundings. The identity $\Delta E = W + Q$ is the first law of thermodynamics, in this discrete-time formulation of the system's microscopic evolution.

The probability to generate a trajectory is the probability to sample the initial microstate x_0, multiplied by the probabilities to generate the subsequent transitions:

$$p_{\lambda_0}^{eq}(x_0) P(x_0 \to x_1; \lambda_1) \cdots P(x_{N-1} \to x_N; \lambda_N). \quad (19)$$

Now let us compare the probability of generating a particular trajectory during the forward process, $P^F[\mathbf{x}]$, and that of generating the time-reversed trajectory during the reverse process, $P^R[\bar{\mathbf{x}}]$, using the convenient shorthand $\mathbf{x} = (x_0 \to \cdots x_N)$ and $\bar{\mathbf{x}} = (x_0 \leftarrow \cdots x_N)$ to specify a *conjugate pair* of trajectories, i.e. a pair related by time-reversal. The ratio of these probabilities is:

$$\frac{p_A^{eq}(x_0)}{p_B^{eq}(x_N)} \frac{P(x_0 \to x_1; \lambda_1^F) \cdots P(x_{N-1} \to x_N; \lambda_N^F)}{P(x_0 \leftarrow x_1; \lambda_{N-1}^R) \cdots P(x_{N-1} \leftarrow x_N; \lambda_0^R)}. \quad (20)$$

Here $(\lambda_0^F, \lambda_1^F, \cdots \lambda_N^F)$ is the protocol for varying the work parameter from A to B during the forward process, and analogous notation is used for the reverse process (R). Since these protocols are related by time-reversal,

$$\lambda_n^R = \lambda_{N-n}^F, \quad (21)$$

we see that every factor $P(x \to x'; \lambda)$ in the numerator of equation (20) is matched by $P(x \leftarrow x'; \lambda)$ in the denominator.

The analysis now reduces to algebraic manipulations. Equations (15), (17), (18), and (20) lead to the simple result, [10]

$$\frac{P^F[\mathbf{x}]}{P^R[\bar{\mathbf{x}}]} = e^{\beta(W^F - \Delta F)}, \tag{22}$$

where $W^F[\mathbf{x}]$ is the work performed on the system during the forward process. If we similarly let W^R denote the work performed during the reverse process, then by equation (17)

$$W^R[\bar{\mathbf{x}}] = -W^F[\mathbf{x}] \tag{23}$$

for a conjugate pair of trajectories, $\mathbf{x}$ and $\bar{\mathbf{x}}$. The work distributions ρ_F and ρ_R are obtained by integrating over all possible discrete trajectories:

$$\rho_F(+W) = \int d\mathbf{x} \, P^F[\mathbf{x}] \, \delta \left(W - W^F[\mathbf{x}] \right) \tag{24}$$

$$\rho_R(-W) = \int d\bar{\mathbf{x}} \, P^R[\bar{\mathbf{x}}] \, \delta \left(W + W^R[\bar{\mathbf{x}}] \right), \tag{25}$$

where $d\mathbf{x} = d\bar{\mathbf{x}} = dx_0 \cdots dx_N$. From equations (22) and (23) it follows that these two distributions satisfy equations (6) [11].

6.3 Feynman-Kac theorem

Hummer and Szabo [24,58] have argued that equation (3) follows from the Feynman-Kac theorem of statistical mechanics [59]. (See also Ge and Jiang [38] for a mathematically rigorous analysis of this issue, and Ao [60] for a discussion in a broader context.) To sketch their derivation, let us assume that the microscopic history of the system is described by continuous-time phase space trajectory, x_t. The system might be thermodynamically isolated during the interval $0 \le t \le \tau$ (in which case x_t obeys Hamilton's equations, as in Sect. 6.1), or it might be in contact with a thermal reservoir. A statistical ensemble of such trajectories, $x_t^1, x_t^2, \cdots, x_t^n, \cdots$, can be described by a time-dependent phase space distribution,

$$f(x, t) = \langle \delta(x - x_t) \rangle, \tag{26}$$

which gives the probability density to find the system at a phase space location x at time t. In this equation x_t is the microstate reached by a particular trajectory at time t, and the angular brackets denote an average over the ensemble of realizations. Under appropriate assumptions [4,24,61], $f(x, t)$ satisfies an equation of motion of the form

$$\frac{\partial f}{\partial t} = \mathcal{L}_t f, \tag{27}$$

where $\mathcal{L}_t$ is a linear operator whose time-dependence enters through the value of the work parameter: $\mathcal{L}_t = \mathcal{L}(\lambda_t)$. For example, if the system evolves under Hamilton's equations, then $\mathcal{L}(\lambda)$ is the Liouville operator,

$\mathcal{L}(\lambda) = (\partial_{\mathbf{p}} H) \, \partial_{\mathbf{q}} - (\partial_{\mathbf{q}} H) \, \partial_{\mathbf{p}}$, where $H = H(x; \lambda)$. Alternatively, if Langevin dynamics are used to model the microscopic motion of the system, then $\mathcal{L}(\lambda)$ includes diffusive terms, in either configuration space or momentum space. We will further assume that this operator "annihilates" the equilibrium distribution, i.e. that

$$\mathcal{L}(\lambda) \exp[-\beta H(x; \lambda)] = 0, \tag{28}$$

for any value of λ. The physical interpretation of this assumption is simple: if the work parameter is held fixed, then the equilibrium distribution is a stationary solution of the dynamics ($f = p^{eq}$ satisfies Eq. (27)). In other words, the system remains in equilibrium unless we actively drive it away from that state by varying λ.

Now consider the function

$$p(x, t) = \frac{1}{Z_A} \exp\left[-\beta H(x; \lambda_t)\right]. \tag{29}$$

Apart from the mismatched normalization factor, this is the equilibrium distribution corresponding to λ_t. By equation (28), p satisfies a "sink equation" [24]

$$\frac{\partial p}{\partial t} = \mathcal{L}_t p - \beta \dot{\lambda} \frac{\partial H}{\partial \lambda} p. \tag{30}$$

The Feynman-Kac theorem states that the solution of such an equation can be represented by an ensemble of trajectories evolving under the original dynamics (Eq. (27)), in which each trajectory x_t carries a time-dependent statistical weight $e^{-\beta w_t}$, analogous to the phase $e^{iS/\hbar}$ appearing in the path-integral formulation of quantum mechanics [62]. In detail, the Feynman-Kac solution of equation (30) is

$$p(x, t) = \left\langle \delta(x - x_t) \, e^{-\beta w_t} \right\rangle, \tag{31}$$

where

$$w_t = \int_0^t ds \, \dot{\lambda} \, \frac{\partial H}{\partial \lambda}(x_s; \lambda_s) \tag{32}$$

is the work performed to time t, for a given realization of the process. Comparing equations (29) and (32) (and assuming an equilibrium distribution at $t = 0$), we arrive at

$$\left\langle \delta(x - x_t) \, e^{-\beta w_t} \right\rangle = \frac{1}{Z_A} \exp\left[-\beta H(x; \lambda_t)\right]. \tag{33}$$

If we integrate both sides with respect to x, and evaluate at $t = \tau$, we once again obtain $\langle e^{-\beta W} \rangle = e^{-\beta \Delta F}$, where $W = w_\tau$ is the work performed over the entire interval.

Beyond providing an alternative derivation of equation (3), (33) reveals that, in principle, if we are presented with an ensemble of microscopic trajectories describing a system driven away from thermal equilibrium, then by the simple trick of endowing each realization with a time-dependent, statistical weight $e^{-\beta w_t}$, we can reconstruct the equilibrium distribution corresponding to the evolving value of λ_t. (See Refs. [24,61,63] for discussions of this point, and Figs. 4–6 of reference [4] for an explicit example of such a reconstruction.)

As an application of this approach, Hummer and Szabo take advantage of equation (33) to construct a method for using non-equilibrium data – e.g. from single-molecule manipulation experiments – to determine equilibrium *potentials of mean force*. These potentials are free energy profiles along a suitably chosen reaction coordinate (such as the end-to-end distance of a stretched polymer molecule, in Ref. [24]), rather than an externally manipulated work parameter, λ.

7 Irreversibility and the second law

I have already remarked that Crooks's fluctuation theorem implies the Kelvin-Planck inequality formulated in equation (5) (see comments following Eq. (6)). In fact there are several interesting connections between nonequilibrium work relations and the second law of thermodynamics.

To begin, note that the relation $\langle e^{-\beta W} \rangle = e^{-\beta \Delta F}$ (Eq. (3)), when combined with $\langle e^x \rangle \geq e^{\langle x \rangle}$ (Jensen's inequality [57]), yields the Clausius inequality, in the statistical form given by equation (2). This might appear to represent a microscopic, first-principles derivation of the second law of thermodynamics, but such a broad interpretation is not warranted. Derivations of equation (3) always begin with the system in a state of thermal equilibrium, generally described by the canonical distribution. This apparently innocent, uncontroversial premise is the Trojan horse by which rather strong assumptions regarding equipartition have been slipped into the analysis from the outset. As Gibbs showed over a century ago, if one is willing to accept the form $p \propto e^{-\beta H}$ as a statistical representation of an equilibrium state, then statements of the second law of thermodynamics (such as the Carnot limit on the work performed by a heat engine, see Eq. (481) of Ref. [64]) follow straightforwardly.

While it is instructive to see that $\langle e^{-\beta W} \rangle = e^{-\beta \Delta F}$ implies $\langle W \rangle \geq \Delta F$, with just a bit more effort, following the derivation of equation (16) of reference [65], we can use equation (3) to obtain a stronger, less expected result (Eq. (37) below). For a given thermodynamic process, let $P[W < \Delta F - \zeta]$ denote the probability to observe a value of work that falls below $\Delta F - \zeta$, where ζ is an arbitrary positive value with units of energy. This probability is simply the area beneath the tail of the distribution $\rho(W)$, to the left of the point $W = \Delta F - \zeta$, so we have

$$P = \int_{-\infty}^{\Delta F - \zeta} dW \, \rho(W) \tag{34}$$

$$\leq \int_{-\infty}^{\Delta F - \zeta} dW \, \rho(W) \, e^{\beta(\Delta F - \zeta - W)} \tag{35}$$

$$\leq e^{-\beta \zeta} \int_{-\infty}^{+\infty} dW \, \rho(W) \, e^{\beta(\Delta F - W)}. \tag{36}$$

By equation (3) the last integrand is unity, hence

$$P[W < \Delta F - \zeta] \leq \exp(-\beta \zeta). \tag{37}$$

Thus the left tail of $\rho(W)$ is exponentially suppressed: while microscopic "violations" of the Clausius inequality

($W < \Delta F$) might occasionally be observed, large violations ($\zeta \gg k_B T$) are effectively forbidden, in agreement with macroscopic experience.

Yet another connection with irreversibility is related to the *conjugate pairing* of realizations: for every trajectory $\mathbf{x}$ that represents a possible microscopic history of the system during the forward process, its time-reversed twin $\bar{\mathbf{x}}$ is a possible realization of the reverse process. (This sort of pairing appeared in Section 6.2 in the context of discrete-time, Markov dynamics, but it applies equally well when the microscopic evolution is modeled by Hamilton's equations [66,67].) Suppose we are shown a movie in which we observe the evolution of some system – say, an optically manipulated biomolecule – as a work parameter λ is varied in the *forward* direction, from A to B. We are then asked to "guess the direction of time", that is, to determine whether: (i) we are watching an actual depiction of the forward process, or whether, instead, (ii) the reverse process was filmed, and now we are being shown that movie, run backward. This is essentially an exercise in statistical inference. Assuming no prior bias in favor of either hypothesis, the likelihood that the observed trajectory originated with the forward process, $L(F|\mathbf{x})$, is proportional to the probability of generating this trajectory when performing that process, $P^F[\mathbf{x}]$; and similarly in the reverse case. Normalizing over the two alternatives (F and R), we have [68,69],

$$L(F|\mathbf{x}) = \frac{P^F[\mathbf{x}]}{P^F[\mathbf{x}] + P^R[\bar{\mathbf{x}}]} = \frac{1}{1 + \exp[-\beta(W - \Delta F)]}, \tag{38}$$

using equation (6). This result conforms to our expectations based on the Clausius inequality: if W greatly exceeds ΔF, then $L(F|\mathbf{x}) \approx 1$, hence we can state with virtual certainty that the movie depicts a realization of the forward process; while in the opposite case we get $L(F|\mathbf{x}) \approx 0$, and we can be equally confident that we are viewing a realization of a reverse process (run backward in time). The transition from one regime to the other occurs over a few $k_B T$ around $W = \Delta F$.

Equation (38) relates dissipated work, $W_{\text{diss}} = W - \Delta F$, to our ability to distinguish between forward and reverse trajectories. Using similar manipulations one can establish a direct connection between physical and information-theoretic measures of irreversibility, namely [67,70]

$$\langle W_{\text{diss}} \rangle_F = k_B T \cdot D[F|R], \tag{39}$$

where on the right side D denotes the *relative entropy* [71] between distributions (in either path space [67] or phase space [70]) representing forward and reverse processes. This result follows by substituting equation (22) (or its continuous-time analogue) into the definition of relative entropy; a similar result for the work distributions ρ_F and ρ_R is an immediate consequence of equation (6). As shown by Parrondo, Kawai, and Van den Broeck [70], equation (39) implies a number of inequalities related to the second law of thermodynamics, such as the Landauer principle on the inherent dissipation that accompanies

information erasure [72]. A result analogous to equation (39) also applies to systems in nonequilibrium steady states [73].

Finally, it is interesting to note that when the left side of equation (3) is evaluated in practice, by taking the average of $e^{-\beta W}$ over many realizations of a given process, the realizations that contribute most to this average are those during which the system appears as though it is evolving backward in time, in a sense made precise in reference [67].

8 Experiments and simulations

To this point, this contribution has focused on theoretical aspects of nonequilibrium work relations. These results, however, have generated considerable interest with respect to their potential applications, in both experimental and computational contexts.

As mentioned above, in 2001 Hummer and Szabo argued that relations such as equation (33) are potentially useful tools for using data obtained from single-molecule manipulation experiments to estimate equilibrium free energy profiles along biologically relevant reaction coordinates [24]. Shortly thereafter, Liphardt et al. [74] published the results of experiments along these lines, demonstrating the practical feasibility of this approach. In these experiments, a single strand of RNA was manipulated using a setup similar to the one depicted schematically in Figure 2. In the absence of external forces, the RNA in this case adopts a single "hairpin" structure. The thermodynamic process in these experiments involved stretching the RNA, causing the structure to unfold. Be repeatedly performing such experiments to obtain a distribution of values of the work required to stretch the molecule, Liphardt et al. provided the first laboratory evidence in support of equation (3). Subsequently, similar RNA-pulling experiments performed by Collin et al. [22] provided confirmation of Crooks's fluctuation theorem.

More recently Harris et al. [75] have used atomic force microscopy to stretch a protein molecule that was constructed as a chain of titin I27 domains, linked in series. The I27 domain is the building block of the giant muscle protein, titin. The protein molecule was attached at one end to a gold substrate on a movable stage, and at the other end to the tip of a microscopic cantilever. The molecule was then stretched by using a piezoelectric actuator to translate the stage, and the displacement of the cantilever from its equilibrium position was monitored as the domains unfolded, one by one. The work W performed in the process of unfolding each domain was then deduced from the force-displacement data, and from these work values the unfolding free energy barrier was obtained using the prescription of reference [24].

(In the time since the submission of this contribution, applications of nonequilibrium work relations to the analysis of single-molecule manipulations have been reported by two other groups [76,77].)

Apart from potential biomolecular applications, nonequilibrium work relations have been probed using a mechanical torsional pendulum, by Douarche et al. [78,79]. In these experiments the torsional thermal fluctuations of a brass wire, suspended in a viscous fluid and with a mirror attached to it, were observed by scattering laser light off the mirror. A time-dependent, oscillatory torque was applied to the brass wire by means of an external magnetic field, thus driving the system away from equilibrium and performing work on it. The statistical distributions of work values were found to satisfy both equations (3) and (6).

These predictions have also been tested in experiments in which optically trapped, micron-size polystyrene beads are first brought to equilibrium with surrounding fluid, then by varying either the position of the focal point or the intensity of the beam, the location or profile of the trap is varied with time. This can take place near a charged surface [80], to produce an asymmetric trap, or in the bulk of the liquid [81]. In either situation, the work W performed on the bead is obtained by monitoring the motion of the bead inside the trap, and the statistical fluctuations of W were again found to agree with equations (3) and (6) [80,81]. Experiments using optically trapped beads have also provided confirmation of the transient fluctuation theorem [2,13], and an analogue of equation (3) pertaining to transitions between nonequilibrium steady states [25,82].

Nonequilibrium work relations also offer a new approach to the numerical estimation of free energy differences, a computationally challenging problem with numerous applications in physics, chemistry and biology [57,83–85]. While traditional free energy estimation methods rely on simulations of systems in thermal equilibrium, equations (3), (6), and (33) reveal that the same information can be obtained from simulations of a system driven away from equilibrium. For instance, reference [86] describes the use of nonequilibrium *steered molecular dynamics* simulations to reconstruct the free energy landscape experienced by an ammonium molecule as it makes its way through a barrel-like protein involved in the regulation of histidine biosynthesis. As with most numerical free energy estimation methods, these techniques suffer from poor convergence, ultimately related to the difficulty of efficiently sampling the relevant regions of phase space; see for instance references [67,87–97] (and references therein) for discussions of these issues and strategies for addressing poor convergence. I will not attempt a comprehensive summary of this currently active area of research. The interested reader can find useful introductions to nonequilibrium-based free energy estimations methods in references [85,98].

9 Summary

In this contribution I have attempted to provide a brief introduction to nonequilibrium work relations, a set of theoretical predictions describing statistical fluctuations in the work performed when driving a system away from equilibrium. The central message here is that these work fluctuations satisfy rather strict laws that we would not reasonably have guessed, simply by extrapolating down from macroscopic experience. These laws are closely related to

the microscopic description of irreversibility. Indeed, in the context considered here they effectively represent what the second law of thermodynamics "looks like", to a microscopic system. Furthermore, they are potentially useful, as they reveal how to extract information about equilibrium properties of a system (e.g. free energy differences) by observing its out-of-equilibrium behavior.

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