

Work, dissipation, and fluctuations in nonequilibrium physics

The nonequilibrium thermodynamics of small systems

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Abstract

Nonequilibrium thermodynamics of small systems describes energy exchange processes between a system and its environment in the low energy range of a few $k_B T$ where Brownian fluctuations are dominant. The main goal of this discipline is to identify the building blocks of a general theory describing energy fluctuations in nonequilibrium processes occurring in systems ranging from condensed matter physics to biophysics. In this article I discuss some aspects about fluctuation theorems and path thermodynamics that call for the necessity of a better understanding of large deviations in nonequilibrium statistical physics. I also emphasize how single molecule experiments are a valuable source of knowledge and information that can greatly contribute to the unveiling of the most important and relevant questions in this exciting field of research. **To cite this article:** *F. Ritort, C. R. Physique 8 (2007).*

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Résumé

La thermodynamique de non-équilibre des petits systèmes. La thermodynamique de non-équilibre des petits systèmes décrit les processus d'échanges d'énergie entre un système et son environnement dans le domaine des basses énergies de quelques $k_B T$ où les fluctuations browniennes sont dominantes. Le but principal de cette discipline est d'identifier les blocs de construction d'une théorie générale décrivant les fluctuations d'énergie dans les processus de non-équilibre se produisant dans des systèmes s'étendant depuis la physique de la matière condensée jusqu'à la biophysique. Dans cet article, je discute quelques aspects des théorèmes de fluctuations et de la thermodynamique des chemins qui nécessitent une meilleure compréhension des grandes déviations en physique statistique de non-équilibre. Je montre aussi comment les expériences sur des molécules individuelles constituent une précieuse source de connaissance et d'information qui peut contribuer grandement à dévoiler les questions les plus importantes et pertinentes dans ce passionnant domaine de recherche. **Pour citer cet article :** *F. Ritort, C. R. Physique 8 (2007).*

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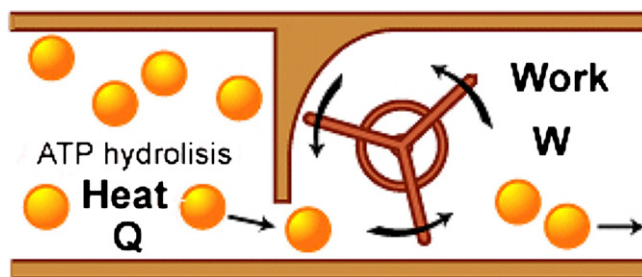


Fig. 1. Illustration of a molecular machine designed to transform part of the energy available from ATP hydrolysis into mechanical work. The efficiency of the machine can be defined as the ratio $\eta = W/\Delta G_{\text{ATP}} < 1$ where W is the maximum amount of work that can be obtained divided by the free energy available from hydrolysis.

1. Motivation

Small systems thermodynamics is a central subject in modern statistical physics. It brings together subjects apparently unrelated, from condensed matter systems to biological molecules. All these systems share the property that energy fluctuations are much larger than $\sim\sqrt{E}$ (the prediction by the law of large numbers) where E is the average energy. Large deviations from average values are normally observed in mesoscopic systems where nonequilibrium fluctuations are governed by a few degrees of freedom. Examples are the Brownian motion of a plastic or silica bead captured in an optical trap, the unfolding of a biomolecule (e.g. a nucleic acid hairpin or protein) or the local reorganization of a frozen region containing a few molecules inside an amorphous material.

In this article I will review some common aspects of these systems that call for the necessity of a general theory capable of describing energy fluctuations in nonequilibrium small systems [1,2]. This necessity is particularly relevant in the case of biological systems which combine an enormous complexity together with a striking efficiency. Molecular motors are a good example in this regard. Molecular motors are large macromolecular assemblies that take advantage of the energy released in many condensation reactions to generate mechanical work (Fig. 1). The precise mechanism by which molecular motors transform the heat released in the hydrolysis into useful work has yet to be elucidated (is it a power stroke or a Brownian ratchet mechanism?) [4,3]. Most of the mechanisms underlying biological function at the molecular level inside the cell operate at high fidelity and efficiency rates under harsh and noisy conditions (environmental Brownian fluctuations, molecular crowding, etc.). This is a singular aspect of biological systems, in contrast to what occurs in other areas of physics and chemistry. Less clear is the need and relevance of such theory to understand old problems in condensed matter physics that have resisted scientific knowledge during many decades. Particularly remarkable is the case of structural glasses where questions posed almost a century ago remain yet unanswered. Is the glass transition a purely kinetic phenomenon? Is there instead a thermodynamic underlying phase transition that is never reached and remains unobservable in laboratory time-scales? What are the *cooperatively rearranging regions* (CRRs) that have been hypothesized to govern relaxational processes in the vicinity of the glass transition region? How can we experimentally observe them in an unambiguous way?

These and other questions point out that our current understanding of nonequilibrium small systems is insufficient. This paper discusses a few selected results on this topic. We will emphasize how single molecule experiments [5] are a valuable source of knowledge and information that can greatly contribute to unveil the most important and relevant questions. Throughout the paper we take $k_B = 1$.

2. Physics and biomolecules

It is a well established fact that the typical amounts of energy obtained from chemical sources (e.g. ATP or GTP hydrolysis) used by most molecular machines are a few kcal per mol [6] (at $T \sim 300K$ this corresponds to a few units of $k_B T$, $1k_B T \simeq 0.6$ kcal/mol). Let us consider the example of RNA transcription. The process by which RNA nucleotides (A,U,G,C) are added to the newly synthesized RNA strand during the transcription process involves the hydrolysis of the different nucleoside-phosphate complexes as they are added to the 3' end of the growing chain. The overall process by which one base is added to the newly synthesized strand is a highly favorable reaction (mainly driven by the hydrolysis of the pyrophosphate) with ΔG in the range between 7 and 12 kcal/mol mainly depending

on the ATP concentration of the environment (e.g. in-vitro versus in-vivo conditions). Effectively this is an irreversible process that generates an amount of available energy between 10 and $20k_B T$ at room temperature (~ 300 K, $1k_B T \simeq 0.6$ kcal/mol) per base pair added. This energy would be lost to the environment in the form of heat were it not for the fact that a big part of the energy is used by the RNA polymerase to locally unwind the double DNA helix and pull apart the two DNA strands to produce a bubble a few bases long of denatured DNA. This bubble is then used by the DNA/RNA/polymerase ternary complex as a substrate to polymerize the RNA. As transcription proceeds the bubble moves downstream together with the RNA polymerase and the RNA transcript is synthesized.

For that process to occur the RNA polymerase must move against the Stokes friction produced by water as well as other roadblocks that hamper its motion. In particular, the RNA polymerase must exert force and torque on the DNA. Typical forces to unzip DNA are on the order of 15 pN meaning that the minimum mechanical work necessary to unzip one base pair is around 15 pN times 12 Å (the typical extension gained after pulling apart two bases at the fork of a DNA hairpin), which is equal to 18 pN nm or equivalently $4.4k_B T$ ($1k_B T \simeq 4.11$ pN nm at room temperature). We can define the efficiency of the RNA motor as the ratio between the mechanical work needed to unzip one base pair and the amount of energy obtained from hydrolysis upon the addition of a nucleotide (however this is not the only way to define mechanical efficiencies, e.g. see [7,8]). The efficiency of the transcription process is then about 40 per cent, a quite remarkable feat if we compare this number with the ones obtained in man made machines (cars have efficiencies below 20 per cent). The motion of a single RNA polymerase has been studied in several prokaryotic systems using optical and magnetic tweezers [9,10]. In these experiments a DNA/polymerase complex is tethered between a trapped bead and an streptavidin coated immobilized bead or surface. To initiate transcription nucleotides are flowed inside the chamber and the elongation of the transcript can be followed in real time while force is applied on the tether. The extension of the RNA transcript as a function of time reveals a complex intermittent motion of the polymerase with pauses (temporary stops), arrests (permanent stops) and even backtracking events [11,12].

3. Fluctuation theorems and path thermodynamics

3.1. Fluctuations in nonequilibrium states

A crucial concept in the statistical description of a nonequilibrium small system is that of a trajectory or path. A path is the time sequence of configurations followed by the system as it is driven to a nonequilibrium state by the action of an external perturbation. Perturbations are usually described in terms of the control parameter λ . These are a set of external parameters (e.g. an electric field, magnetic field, optical force, ...) that can be experimentally controlled and do not fluctuate. Experimentally, control parameters are produced by macroscopic systems that are used to manipulate the small system under study and which are insensitive to thermal fluctuations (but that produce other sort of uncontrolled instrumental noises and drift effects).

There are infinite ways to generate nonequilibrium states. Particularly important are transient nonequilibrium states. These are generated by taking a system that is initially equilibrated and driving it out of equilibrium by fast switching of an external perturbation. Systems embedded in a thermal environment follow different trajectories if the same experiment is repeated under identical conditions (according to a given protocol). By repeating the same experiment many times the value of the work W done on the system changes from path to path allowing us to define its probability distribution, $P(W)$. In macroscopic systems Brownian fluctuations often induce unobservable effects on the properties of the system (fluctuations in the vicinity of critical points and instabilities in far from equilibrium systems are exceptions). However, in small systems, Brownian fluctuations can be easily detected and measured with good accuracy with the aid of recently developed micromanipulation tools.

The second law of thermodynamics puts bounds on the minimum amount of average work performed on the system: although W may strongly fluctuate from path to path its mean value (averaged over an infinite number of repeated experiments, i.e. the first moment of $P(W)$) is always greater than the reversible or quasi-static work, W_{rev} , which is also equal to the free energy difference ΔF between the initial and final equilibrium states. The reversible work is the value of the work that is obtained for protocols that are adiabatic or quasi-static, i.e. the control parameters are changed infinitely slowly. The difference between the actual work and the reversible work is called the dissipated work, $W_{\text{diss}} = W - \Delta F$. The second law establishes that in average a positive amount of heat is irreversibly lost to the environment, $\overline{W_{\text{diss}}} \geq 0$, where $\overline{(\dots)}$ stands for the average taken over an infinite number of paths. The amount of

dissipation in irreversible processes is then related to the asymmetry between the phase space densities obtained when the process is run forward and backward in time [13].

Several results known under the generic name of fluctuation theorems (FTs) have been recently developed that put more stringent conditions on the spectrum of work fluctuations in a nonequilibrium experiment, the second law being just a particular consequence of such results [14–21]. Prominent examples are the nonequilibrium work equality by Jarzynski [22] and the fluctuation relation by Crooks [23,24] that allow one to recover free energy differences from measurements of the irreversible work along nonequilibrium processes. These relations have been demonstrated under a few assumptions and for a large variety of nonequilibrium theories and solvable models (see for instance [25,26]). They read,

$$\overline{\exp\left(-\frac{W_{\text{diss}}}{T}\right)} = 1 \quad \text{or} \quad \overline{\exp\left(-\frac{W}{T}\right)} = \exp\left(-\frac{\Delta F}{T}\right) \quad (1)$$

$$\frac{P_F(W_{\text{diss}})}{P_R(-W_{\text{diss}})} = \exp\left(\frac{W_{\text{diss}}}{T}\right) \quad \text{or} \quad \frac{P_F(W)}{P_R(-W)} = \exp\left(\frac{W - \Delta F}{T}\right) \quad (2)$$

In (2) the $P_F(W)$, $P_R(W)$ denote the forward and reverse work distributions corresponding to the forward and reverse processes (time reversed images of each other). The Crooks relation (2) provides a way to determine free energy differences from measurements of the irreversible work by looking at the work value where forward and reverse work distributions cross each other. According to (2), $P_F(W = \Delta F) = P_R(-W = -\Delta F)$.

The second law of thermodynamics, $\overline{W} \geq \Delta F$, also follows naturally as a particular case of (1) by using the convexity inequality, $\overline{\exp(x)} \geq \exp(\overline{x})$. Gaussian work distributions exactly satisfy (2) provided the average dissipated work $\overline{W}_{\text{diss}}$ and the variance of the work, σ_W^2 , are identical along the forward and reverse processes and $\sigma_W^2 = 2k_B T \overline{W}_{\text{diss}}$. A fluctuation–dissipation parameter R can be introduced to quantify deviations from the Gaussian behavior [27],

$$R = \frac{\sigma_W^2}{2k_B T \overline{W}_{\text{diss}}} \quad (3)$$

For Gaussian work distributions $R = 1$, corresponding to nonequilibrium processes in the linear regime where the fluctuation–dissipation theorem holds.

3.2. Path thermodynamics

One of the consequences of (1) is the possibility to define thermodynamic potentials associated to paths or trajectories [28]. We define the *path entropy* $S(W)$ as the logarithm of the density of paths with work equal to W , $P(W) = \exp(S(W))$. Eq. (1) can be rewritten as,

$$\begin{aligned} \exp\left(-\frac{\Delta F}{T}\right) &= \sum_{\Gamma} \exp\left(-\frac{W(\Gamma)}{T}\right) = \int dW P(W) \exp\left(-\frac{W}{T}\right) \\ &= \int dW \exp\left(S(W) - \frac{W}{T}\right) = \int dW \exp\left(-\frac{\Phi(W)}{T}\right) \end{aligned} \quad (4)$$

where Γ denotes an arbitrary path and $\Phi(W) = W - TS(W)$ is the *path free energy*. In the large volume limit the sum in (4) is dominated by the work value, W^\dagger , where $\Phi(W)$ is minimum. We note from (4) that the path free energy $\Phi(W^\dagger)$ is equal to the free energy difference ΔF . The following chain of relations hold,

$$\Delta F = \Phi(W^\dagger) = W^\dagger - TS(W^\dagger) \quad (5)$$

$$\left(\frac{\partial \Phi(W)}{\partial W}\right)_{W=W^\dagger} = 0 \rightarrow \left(\frac{\partial S(W)}{\partial W}\right)_{W=W^\dagger} = \frac{1}{T} \quad (6)$$

At the same time, W^\dagger is different from the most probable work, W^{mp} , defined as the work value at which $S(W)$ is maximum,

$$\left(\frac{\partial S(W)}{\partial W}\right)_{W=W^{\text{mp}}} = 0 \rightarrow \left(\frac{\partial \Phi(W)}{\partial W}\right)_{W=W^{\text{mp}}} = 1 \quad (7)$$

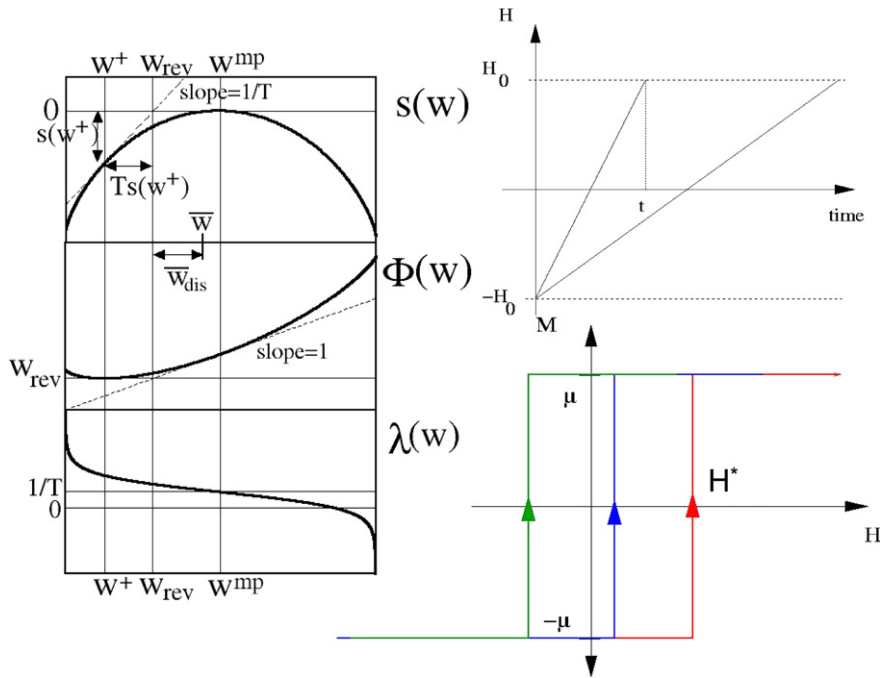


Fig. 2. Upper left panel: Path entropy $s(w)$. Middle left panel: Path free-energy $\Phi(w) = w - Ts(w)$. Lower left panel: Lagrange multiplier $\lambda(w)$ equal to the inverse of the path temperature $1/\hat{T}(w)$. Figure taken from [28]. (Upper right) Ramping protocol. (Lower right) Three possible one-transition paths of a dipole. The dipole reverses orientation at different values of the field H^* .

The path entropy is associated to a *path temperature*, $\hat{T}(W)$,

$$\frac{\partial S(W)}{\partial W} = \lambda(W) = \frac{1}{\hat{T}(W)} \rightarrow \hat{T}(W^\dagger) = T \quad (8)$$

where $\lambda(W)$ is a Lagrange multiplier that transforms the path entropy $S(W)$ into the path free energy $\Phi(W)$. The mathematical relations between the quantities W^\dagger and W^{mp} can be graphically represented for a given path entropy $S(W)$ (see left panels in Fig. 2). The path thermodynamics formalism allows us to extract some general conclusions on the relation between W^\dagger and W^{mp} . In terms of the path entropies for the forward and reverse processes, $S_F(W)$ and $S_R(W)$, (2) can be written as,

$$S_F(W) - S_R(-W) = \frac{W - \Delta F}{T} \rightarrow (S_F)'(W) + (S_R)'(-W) = \frac{1}{T} \quad (9)$$

where we used the definition $S(W) = \log(P(W))$ and later derived with respect to W . By inserting $W = W_F^\dagger$ and $W = -W_R^\dagger$ in the rhs of (9) and using (6), (7) we obtain the following chain of relations,

$$(S_F)'(W_F^\dagger) + (S_R)'(-W_F^\dagger) = \frac{1}{T} \rightarrow (S_R)'(-W_F^\dagger) = 0 \rightarrow W_F^\dagger = -W_R^{\text{mp}} \quad (10)$$

$$(S_F)'(-W_R^\dagger) + (S_R)'(W_R^\dagger) = \frac{1}{T} \rightarrow (S_F)'(-W_R^\dagger) = 0 \rightarrow W_R^\dagger = -W_F^{\text{mp}} \quad (11)$$

The rightmost equalities in (10), (11) imply that the most probable work along the forward (reverse) process is equal to the work value (W^\dagger) that must be sampled, in a finite number of experiments, along the reverse (forward) process for the Jarzynski equality to be satisfied. The process among the forward and reverse with largest dissipation is the one that samples best the region of values in the vicinity of W^\dagger . This conclusion that may look counterintuitive can be rationalized by noting that larger dissipation implies also larger fluctuations and therefore more chances to get rare paths that sample the vicinity of W^\dagger . The symmetries (10), (11) were originally discussed in [28] and analyzed in detail for the case of the gas contained in a piston [29].

3.3. An example

As an example of the previous set of relations let us consider a magnetic dipole subject to an external field H . The dipole can take two possible values $\mu\sigma$ with $\sigma = \pm 1$, the energy of the dipole being $E = -\mu\sigma H$. The transition rates between the up and down orientations are of the Kramers type [32,33],

$$k_{-\mu \rightarrow \mu}(H) = k_{\text{up}}(H) = k_0 \frac{\exp(\frac{\mu H}{T})}{2 \cosh(\frac{\mu H}{T})} \tag{12}$$

$$k_{\mu \rightarrow -\mu}(H) = k_{\text{down}}(H) = k_0 \frac{\exp(-\frac{\mu H}{T})}{2 \cosh(\frac{\mu H}{T})} \tag{13}$$

with $k_0 = k_{\text{up}}(H) + k_{\text{down}}(H)$ independent of H . The rates (12), (13) satisfy detailed balance,

$$\frac{k_{\text{up}}(H)}{k_{\text{down}}(H)} = \frac{P^{\text{eq}}(\mu)}{P^{\text{eq}}(-\mu)} = \exp\left(-\frac{2\mu H}{T}\right) \tag{14}$$

with $P^{\text{eq}}(+(-)\mu) = \exp(-(+)\mu H/T)/Z$ where $Z = 2 \cosh(\mu H/T)$ is the equilibrium partition function. We now consider a transient nonequilibrium state produced in a ramping experiment where the magnetic field $H(t)$ is varied at a constant speed $r = \dot{H}$. The system starts from an initially equilibrated state at the initial value of the field, $H = -H_0$, and ends at $H = H_0$. For simplicity we take the limit $H_0 \rightarrow \infty$ by keeping the ramping speed r finite. The work done by the ramping field on the dipole along a given path is given by,

$$W(\Gamma) = - \int_{-\infty}^{\infty} dt \dot{H}(t) \mu(t) = -r \int_{-\infty}^{\infty} dt \mu(t) \tag{15}$$

Note that because $E_H(\mu) = E_{-H}(-\mu)$ then $\Delta E = 0$ and $Q(\Gamma) = W(\Gamma)$ so heat and work are identical quantities in this example. Moreover, due to the time reversal symmetry of the ramping protocol, the work distribution $P(W)$ is identical along the forward and reverse processes, $P_F(W) = P_R(W)$.

The exact computation of $P(W)$ in this simple one-dipole model is already a very arduous task. We can, however, consider a limiting case and try to elucidate the properties of the work (heat) distribution. Here we consider the limit of large ramping speed r where the dipole executes just one transition from the down to the up orientation. A few of these paths are depicted in the lower right panel of Fig. 2. In this reduced and oversimplified description a path is fully specified by the value of the field H^* at which the dipole reverses orientation. The work along one of these paths is given by,

$$W(\Gamma \equiv H^*) = - \lim_{H_0 \rightarrow \infty} \int_{-H_0}^{H_0} dH \mu(H) = ((H^* + H_0) - (H_0 - H^*))\mu = 2\mu H^* \tag{16}$$

According to the second law, $\overline{W} = \overline{Q} \geq 0$, which implies that the average switching field is positive, $\overline{H^*} \geq 0$ (as expected due to the time lag between the reversal of the field and the reversal of the dipole). The work distribution is just given by the switching field distribution $p(H^*)$. This is a quantity easy to compute. The probability that the dipole is in the down state at field H satisfies a master equation that only includes a *death* term,

$$\frac{\partial p_{\text{down}}(H)}{\partial H} = -\frac{k_{\text{up}}(H)}{r} p_{\text{down}}(H) \tag{17}$$

This equation can be exactly solved to yield,

$$p_{\text{down}}(H) = \exp\left(-\frac{1}{r} \int_{-\infty}^H dH k_{\text{up}}(H)\right) = \left(1 + \exp\left(\frac{2\mu H}{T}\right)\right)^{-\frac{T k_0}{2\mu r}} \tag{18}$$

Using the relation $P(H^*) = -(p_{\text{down}})'(H^*)$ and (16) we get,

$$P(W) = \frac{k_0}{4\mu r} \left(1 + \exp\left(\frac{W}{T}\right)\right)^{-\frac{T k_0}{2\mu r}} \frac{\exp(\frac{2W}{T})}{\cosh(\frac{2W}{T})} \tag{19}$$

and from this result we obtain the path entropy,

$$S(W) = \log(P(W)) = -\frac{Tk_0}{2\mu r} \log\left(\exp\left(\frac{W}{T}\right) + 1\right) + \frac{W}{2T} - \log\left(\cosh\left(\frac{W}{2T}\right)\right) + \text{constant} \quad (20)$$

It is important to stress that (19) does not satisfy (2) (with $P_F(W) = P_R(W)$) except in the limit $r \rightarrow \infty$ where this approximation becomes exact. We now consider the large r limit. For W^{mp}, W^\dagger we obtain to the leading order,

$$S'(W^{\text{mp}}) = 0 \rightarrow W^{\text{mp}} = T \log\left(\frac{2\mu r}{k_0 T}\right) + O\left(\frac{1}{r}\right) \quad (21)$$

$$S'(W^\dagger) = \frac{1}{T} \rightarrow W^\dagger = -T \log\left(\frac{2\mu r}{k_0 T}\right) + O\left(\frac{1}{r}\right) \quad (22)$$

so the symmetry (10) (or (11)) is satisfied to the leading order in r (yet it can be shown that the $1/r$ corrections appearing in W^{mp}, W^\dagger (21), (22) are different). The logarithmic increase of the average work with the ramping speed (21) is just a consequence of the logarithmic increase of the average value of the switching field $\overline{H^*}$ with the ramping speed. This result has been also found for the dependence of the average breakage force of molecular bonds in single molecule pulling experiments. This phenomenology, related to the technique commonly known as dynamic force spectroscopy, allows to explore free energy landscapes by varying the pulling speed over several orders of magnitude [34,35].

3.4. Large work/heat deviations

The work distribution obtained in the previous example (hereafter we use $q = Q = W$) shows the presence of large work/heat tails. For $|q| \rightarrow \infty$ we get,

$$s(q \rightarrow \infty) = -\frac{qk_0}{2\mu r} + O\left(\exp\left(-\frac{q}{T}\right)\right) \quad (23)$$

$$s(q \rightarrow -\infty) = \frac{q}{T} + O\left(\exp\left(\frac{q}{T}\right)\right) \quad (24)$$

The linear dependence of $s(q)$ on q leads to

$$\hat{T}(q \rightarrow \infty) = T^- = -\frac{2\mu r}{k_0} \quad (25)$$

$$\hat{T}(q \rightarrow -\infty) = T^+ = T \quad (26)$$

where we use the notation T^+, T^- to stress the fact that these path temperatures are positive and negative respectively. Both path temperatures are constant and lead to exponential tails for positive and negative work values. Note that Eq. (9) reads,

$$s(q) - s(-q) = \frac{q}{T} \rightarrow (s)'(q) + (s)'(-q) = \frac{1}{T} \rightarrow \frac{1}{T^+} + \frac{1}{T^-} = \frac{1}{T} \quad (27)$$

which is satisfied by (25), (26) up to $1/r$ corrections. Fig. 3 shows the results obtained for the path entropy, free energy and temperature using a mean-field path integral formalism that includes arbitrary paths with more than one reversal of the dipole (for details of the computations see [28,30]). A more detailed analysis [31] has shown that a plateau is never fully reached for a finite interval of heat values when $r \rightarrow 0$. The presence of a plateau has been interpreted in terms of a first order phase transition appearing in the path entropy $s(q)$ [31].

The path entropy $s(w)$ contains two fluctuation sectors [2] (see also [36] for a discussion of these sectors in the context of oscillator models for glassy dynamics):

- *The FDT or stimulated sector*: This sector is described by Gaussian work fluctuations leading to $s(q) = -(q - q^{\text{mp}})^2 / (2\sigma_q^2) + \text{constant}$. Therefore we get, from (8), $\lambda(q) = \frac{1}{\hat{T}(q)} = -\frac{q - q^{\text{mp}}}{\sigma_q^2}$ showing a linear dependence in q for small deviations around q^{mp} . Note that $\hat{T}(q)$ satisfies (27) and therefore $\sigma_q^2 = 2Tq^{\text{mp}}$, leading to a fluctuation-dissipation parameter $R = 1$ (3). This sector we call stimulated because the magnitude of heat fluctuations is governed by the temperature of the bath.

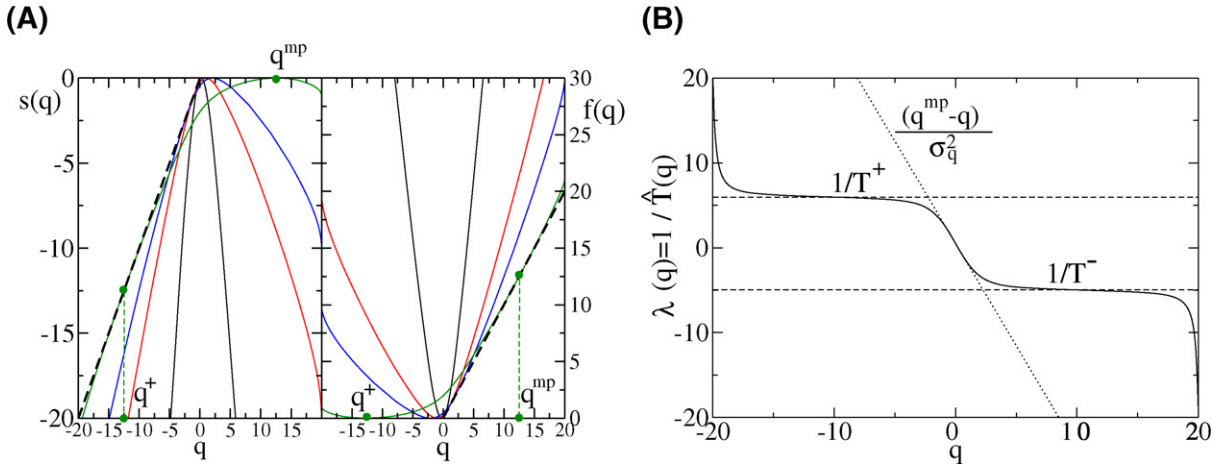


Fig. 3. (A) Heat distributions (path entropy $s(q)$ and path free energy $f(q)$) evaluated at four values of increasing ramping speeds (from the most narrower to the most wider distributions). The dashed line in the left panel is $y(q) = q/T$ (we take $T = 1$) and is tangent to $s(q)$ at q^+ (filled dots shown for the largest ramping speed). The dashed line in the right panel corresponds to $y(q) = q$ and is tangent to the function $f(q)$ at the value q^{mp} (filled dots are shown for the largest speed). (B) $\lambda(q)$ for the lowest speed. It shows a linear behavior for small values of q (dotted line). Figure adapted from [28].

– *The large deviation or spontaneous sector:* Under some conditions this sector is well reproduced by exponential tails in the work distribution describing large or macroscopic deviations. In this sector,

$$\frac{1}{\hat{T}(q)} - \frac{1}{\hat{T}(-q)} = \frac{1}{T} \rightarrow \frac{1}{T^+} + \frac{1}{T^-} = \frac{1}{T} \quad (28)$$

The physical interpretation of T^+ , T^- is as follows. Because $T^- < 0$ then T^- describes fluctuations (corresponding to q positive and large (25)) where net heat is released from the system to the bath, whereas $T^+ > 0$ describes fluctuations (corresponding to q negative and large (26)) where the system absorbs a net amount of heat from the bath. Eq. (28) imposes $T^+ < |T^-|$ implying that large deviations also satisfy the second law: the average net amount of heat supplied to the bath ($\propto |T^-|$) is always larger than the average net heat absorbed from the bath ($\propto T^+$). In the previous example (25), (26), T^+ converges to the bath temperature whereas T^- diverges to $-\infty$ when $r \rightarrow \infty$. We call this sector spontaneous because the energy fluctuations mainly depend on the nonequilibrium protocol (in the present example, such dependence is contained in the r -dependence of T^- , (25)).

4. Free energy recovery in single molecule experiments

Single molecule force experiments [5] using optical tweezers have provided some of the first experimental tests of fluctuation theorems. The basis of single force measurements is to use a local probe (a cantilever in AFM, a glass bead in optical tweezers, a magnetic bead in magnetic tweezers, etc.) to detect forces in the pN range. The sensitivity in force and extension of these probes is limited by Brownian fluctuations which put upper bounds to the resolution of the instrument. In general, force and extension sensitivity are determined by the magnitude of the fluctuations $\Delta f \sim \sqrt{k_B T k}$ and $\Delta x \sim \sqrt{k_B T / k}$ where k is the effective stiffness or rigidity of the probe (a combination the stiffness of the probe and the stiffness of the molecular system that is manipulated). A large value of k implies high spatial resolution but low force resolution (a good example is the AFM which due to its high spatial resolution is also used as an imaging tool) whereas at low k values we have low spatial but high force resolution (e.g. magnetic tweezers). In general, $\Delta f \Delta x \sim k_B T$ and accuracies in both quantities appear to be complementary, a sort of uncertainty principle. Optical tweezers offer a good balance between both limiting cases with Δf and Δx both reasonably small (in the 0.1 pN and 1 Å ranges respectively).

Optical tweezers are based on the optical gradient force discovered by Ashkin in 1970. By focusing an infrared laser beam (to avoid heating effects in water) and using a water or oil immersed objective with high numerical aperture

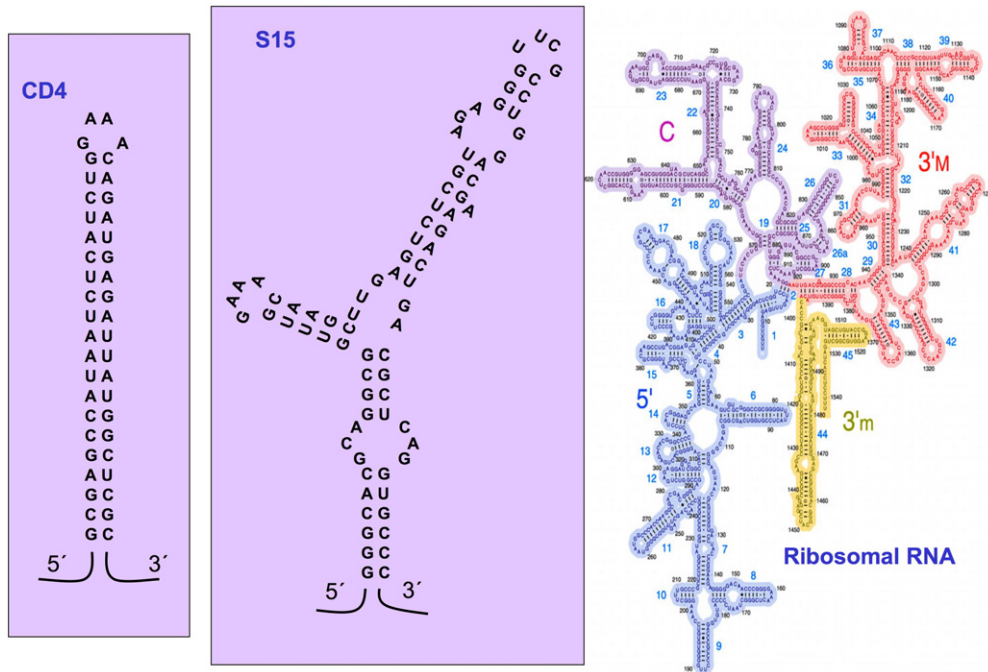


Fig. 4. Three RNA molecules: CD4, a canonical hairpin (left); S15, a three way junction (center); and a subunit of ribosomal RNA in *E. coli* (right). FTs have been tested in the first two molecules.

(in the range 1.2–1.4) it is possible to trap micron sized beads having an index of refraction higher than that of water (e.g. of polystyrene or silica beads). In order to reduce the scattering force along the optical axis it is useful to build optical tweezers with counter propagating laser beams. In this setup it is then possible to use objectives with numerical aperture in the lower range (≈ 1.2) and, at the same time, collect all the deflected light by the bead. This allows to measure the transverse and axial components of the force acting on the bead by using position sensitive detectors located at opposite sides of each laser beam.

Over the past years RNA has been investigated using optical tweezers. There are several reasons that make RNA a good model system to investigate nonequilibrium fluctuations in small systems. RNA is a very important player in molecular biology that shows biological functions in between those attributed to DNA and proteins. For the biophysicist and the statistical physicist RNA is also a fascinating molecule. Primarily found in nature in single stranded form, RNA folds into a three dimensional structure mainly stabilized by stacking interactions and hydrogen bonds between complementary bases (A-U,G-C). Full complementarity between different RNA segments is often impossible so, at difference with DNA, RNA structure also includes mismatches between bases as well other structural defects (bulges, loops, junctions, ...). In addition to Watson-Crick base pairing, RNA forms a compact structure through specific interactions mediated by magnesium ions that bring together distal RNA segments. A few structures where FTs have been tested are shown in Fig. 4. Another aspect that makes RNA particularly interesting, at difference with proteins, is its modular structure (Fig. 4). This fact makes RNA particularly suited to investigate questions related to the folding of biomolecules. How does RNA fold? Does folding occur majoritarily in one big step where all domains are simultaneously stabilized and the entropy of formation is suddenly released as heat to the environment? Or, instead, does folding occur in a series of steps, where at each step a few kcal per mol are dissipated to the environment? Understanding the process by which molecules dissipate energy while they fold may shed light on the physical mechanisms behind the selection of specific molecular structures during evolution.

RNA molecules can be manipulated using optical tweezers and the folding/unfolding kinetics investigated under mechanical force [37]. From the force extension curves it is then possible to measure the work done upon/by the molecule by/on the instrument [38]. In order to pull RNA molecules it is useful to insert them between double stranded DNA/RNA hybrid handles that provide the necessary space between the micron sized beads to carry out the manipulation (Fig. 5A). The whole molecular construct (RNA plus handles) can be synthesized using standard gene cloning

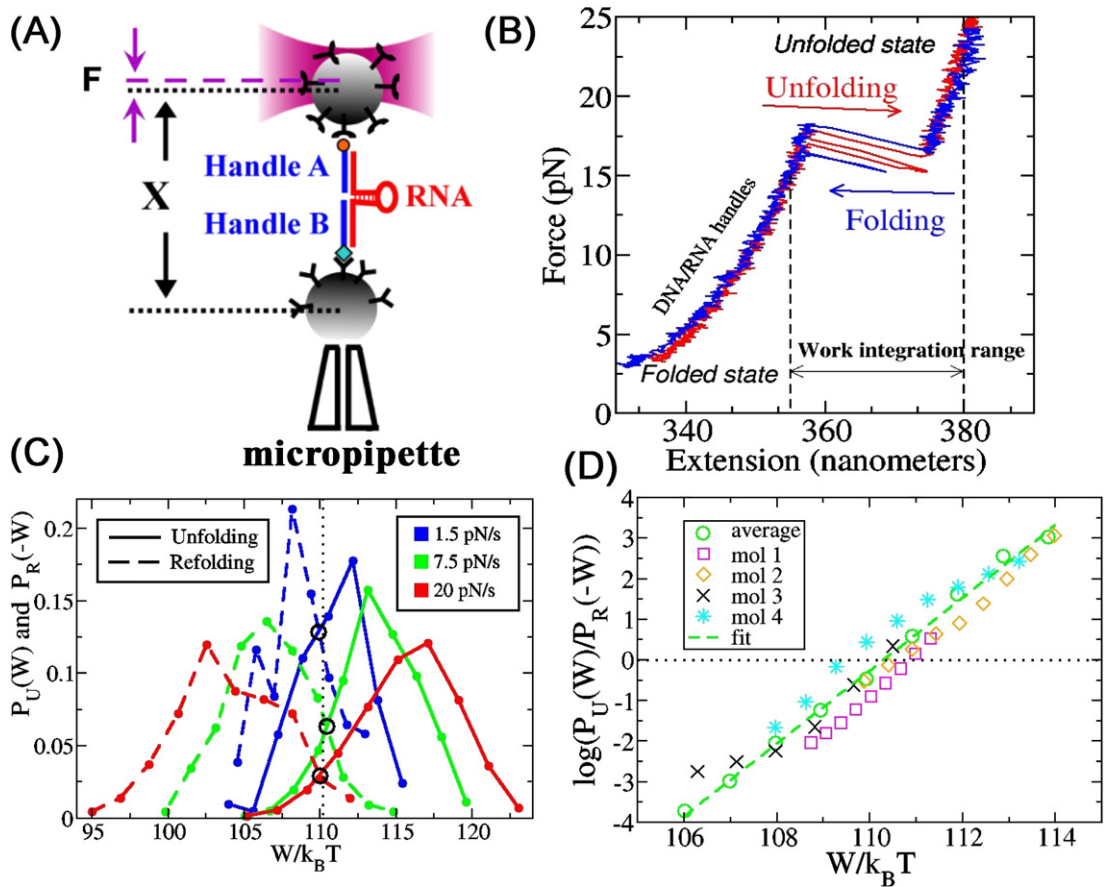


Fig. 5. (A) Experimental setup in RNA force pulling experiments. (B) Force-extension curves in the canonical hairpin CD4 showing the work integration range. (C) Unfolding/refolding work distributions measured at three pulling speeds. (D) Verification of the Crooks FT. Results taken from [39].

and PCR techniques. In such experiments RNA molecules can be repeatedly pulled and relaxed to produce unfolding and refolding force-extension curves such as those shown in Fig. 5B for the canonical hairpin CD4. By measuring the area under the curve it is then possible to estimate the work done on/by the molecule along each unfolding/refolding path. Work distributions for different molecules and different speeds are shown in Fig. 5C and the test of the FT in Fig. 5D. As predicted by the FT (3), work distributions cross at the same value of the work for all three pulling speeds. From this value we can extract the free energy of formation of the hairpin, ΔF , after subtracting the elastic contributions due to the handles and the ssRNA. The canonical hairpin does not show too irreversible behavior at the pulling speeds investigated (around 100 nm/s) and dissipated work values are not big (around $6k_B T$ at 500 nm/s). In contrast, the force-extension curves for the three way junction S15 shown in Fig. 6 show strong irreversibility effects (e.g. hysteresis) as expected for a larger molecule. S15 unfolds in a cooperative way but refolds less cooperatively and over longer timescales. The amount of dissipated work is much larger (around $50k_B T$ for the unfolding and around $20k_B T$ during the refolding). The FT has been experimentally verified in the case of a mutant of S15 (differing from the wild type in a single base pair) that folds faster than the wild type. Forward and reverse distributions cross at values of ΔF that differ in $3k_B T$ which is equal to the difference in free energy of formation between the mutant and the wild type.

5. Conclusions

The experimental and theoretical study of nonequilibrium small systems offers exciting possibilities for the statistical physicist and the biophysicist. This discipline aims to describe the novel properties observed in biomolecules

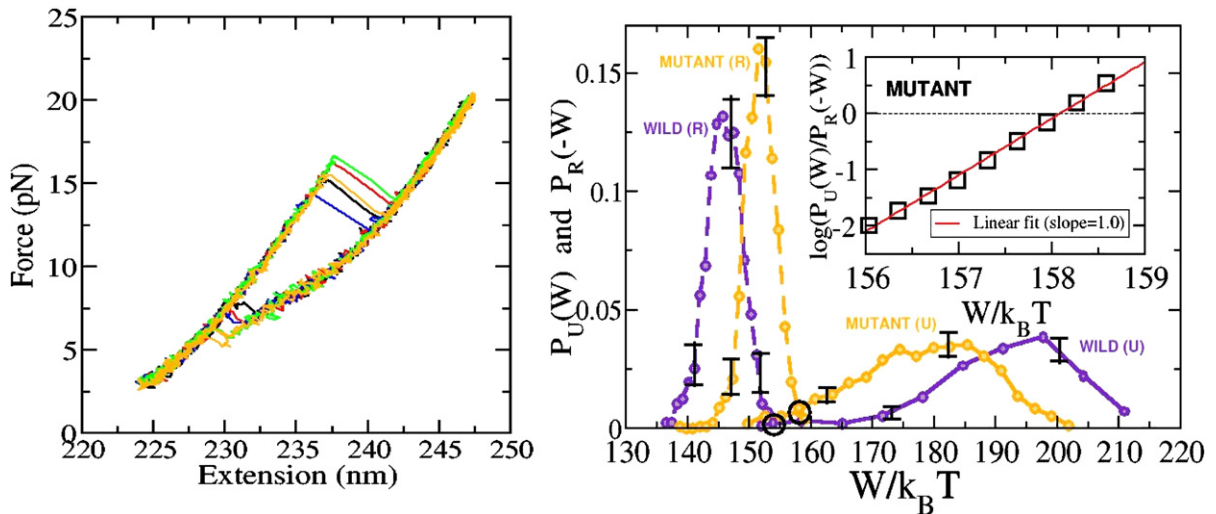


Fig. 6. (Left) Force-extension curves in the three way junction S15 (wild type). (Right) Work distributions measured for the wild type and a mutant differing in one base pair swap ($GC \rightarrow CG$) located in the stem just below the junction. In the inset we show the experimental verification of the Crooks FT. Results taken from [39].

and molecular machines operating far from equilibrium, such as the folding of a nucleic acid or a protein or the translocation motion of a molecular motor.

We are just starting to take a glance at how these small objects exchange energy with their environment. It is a well known fact in molecular biology and biochemistry that biological function at the molecular level is tightly related to structure. It might not be surprising that the link between molecular structure and biological function is encoded in the low frequency region of the spectrum of nonequilibrium energy fluctuations (the spectrum of energy fluctuations extending far at the most extreme tails of the distribution). It is difficult to imagine how biomolecular processes, often carrying a lot of information, can operate solely from high frequency events describing the motion of a few number of atoms. Rather, these should somehow rely on the low frequency cooperative motion between different and distant parts of the molecule. In other words, nonequilibrium fluctuations that drive biological function must be encoded in the tails of the energy exchange distribution, what we have called the spontaneous sector here and in previous works (see [2] for a review). Investigating the spontaneous sector of fluctuations in nonequilibrium systems calls for a deeper theoretical understanding of large deviation functions in nonequilibrium systems (see for instance [40]) as well as more systematic and accurate experiments identifying sources of large energy fluctuations in biological systems.

We are at the dawn of an interdisciplinary scientific discipline that will bring together scientists with expertises coming from very different branches of knowledge. This merging process might culminate with the future engineering of artificial mesoscopic structures capable of reproducing and even improving the behavior of the biological ones.

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