The physics of small systems: From energy to information

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Summary. The focus of this review is the recent developments in the non-equilibrium physics of small systems. Special emphasis is placed on single-molecule experiments and their contribution to expanding our current understanding of fundamental concepts, such as temperature, energy, entropy, and information. [**Contrib Sci** 11(2): 137-146 (2015)]

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Non-equilibrium, dissipation, and entropy production

Non-equilibrium conditions pervade nature. From a waterfall to a star, from a microbe to a human being, all natural systems are intrinsically non-equilibrium, as equilibrium systems are only an approximate description of what we observe (but indeed a very good one in some cases). What is the key signature of non-equilibrium systems? In general, we can say that a system is out of equilibrium when there are net currents across the system of any conserved quantity, such as mass, charge, momentum, and energy. For example, consider a block of mass sitting on the floor. If we move the block by pulling on it, then frictional forces between the block and the floor arise that heat up the block at the contact area. A net amount of energy in the form of heat then flows from the block to the floor. The block is out of equilibrium. In yet another example, an electric current flowing through a resistance (e.g., a metal) heats up the resistance due to the frictional forces generated by the collisions between the moving electrons and the metal atoms of the resistance. The heat generated in the resistance is then dissipated to the environment, resulting in a net heat flux.

In both examples, an external agent (a mechanical force for the block and an electric field for the current) exerts power on the system that results in the delivery of heat Q to the environment and a corresponding increase in entropy given by Q/T, where T is the temperature of the environment. Physicists define this increase as positive entropy production. Broadly speaking, non-equilibrium systems are those in which entropy is produced whereas for equilibrium systems entropy production equals zero. Entropy production is strictly positive and the heat produced in the previous examples is always dissipated to the surroundings. If the opposite is observed, for example, if heat spontaneously flows from the environment to the resistance, generating a net electric current, entropy production is then negative. Such events are rare, their occurrence being interpreted as evidence that somewhere else in the universe entropy must have been produced to compensate for the decrease, because the global balance of entropy production in the universe is always positive.

However, closer examination shows more complex behaviors than heat flowing from hot to cold. Suppose we have an experimental device capable of measuring how much heat is delivered to the surroundings during a given amount of time. If the time window were long enough, then we would observe that average entropy production is always positive. However, if the time window was decreased below a characteristic timescale, then entropy production would sometimes be positive and other times negative, meaning that heat ocassionally flows from the colder surrounding to the hotter body. In this case, the amount of heat transferred fluctuates from measurement to measurement, not only in magnitude but also in sign. The shorter the time window, the stronger the heat fluctuations and the more probable such negative events are. But what is the origin of these fluctuations?

In 1827, Robert Brown, a botanist well-known for his detailed descriptions of the cell nucleus and cytoplasm and for his contributions to the taxonomy of plants, made an important discovery. During microscopy observations of the grains of pollen of a plant suspended in water, he noticed that their motion was erratic and unpredictable, as if the grains were alive. It was only after the validation of the atomic hypothesis at the beginning of the 20th century that it became clear that what Brown had observed was the effect of the stochastic or random collisions of the molecules of water against the grains of pollen. Bombarded from all directions, the suspended grains of pollen jiggled erratically. These conclusions were supported by the Smoluchovsky-Einstein theory of Brownian motion, in 1905. Later experiments conducted by Jean-Baptiste Perrin on diffusive colloidal particles provided the final

proof. Perrin was also able to accurately estimate Avogadro's number using physical methods alone; his results agreed with those reported by chemists. Statistical physics, the appropriate theoretical ground for thermodynamics, builds on the atomistic nature of matter and the probabilistic nature of heat and work. Today, not only has the probabilistic feature been acknowledged by scientists, it is also fundamental to our current understanding of non-equilibrium thermodynamics.

When an electric current flows through a resistance, heat is generated (and entropy produced). However, the moving cloud of electrons experiences Brownian forces that lead to voltage fluctuations across the resistance. This effect is commonly known as Nyquist noise and was first experimentally observed by John Bertrand Johnson at Bell Labs 1926. For time windows that are comparable to the decorrelation time of the voltage signal, the heat and entropy produced are positive most of the times (the cloud moves in the direction of the electric field), but occasionally heat and entropy production are negative (the electron cloud moves against the field). The latter are rare events, marked by the flow of heat from the environment to the resistance and its conversion into work to move the electron cloud against the field. It is important to stress that, despite large fluctuations, average entropy production is always positive, which ultimately constitutes the core of the second law of thermodynamics.

Under which conditions are fluctuations in entropy production experimentally observable? For a system of N degrees of freedom, extensive quantities, such as entropy production or the energy content, grow linearly with N while the size of the fluctuations scale according to $\sqrt{N}$ based on the law of large numbers. The relative magnitude of these fluctuations then decays as $1/\sqrt{N}$, indicating that they are measurable if N it is not too large. According to the equipartition law, each degree of freedom contributes to the average energy by an amount roughly equal to $k_B T$, where k_B is the Boltzmann constant and T is the temperature. Entropy production and energy fluctuations are measurable if the energies involved are a few $k_B T$, meaning that the energies delivered to the system by the external agent are not too high and are comparable to the average kinetic energy carried by the colliding molecules in the thermal environment (on the order of $k_B T$). These are the so-called small systems and the branch of physics devoted to the study of the energy transformation processes in them under non-equilibrium conditions is referred as the *non-equilibrium thermodynamics of small systems*, or *stochastic thermodynamics* [4,13,18,21].

Examples of small systems that have been experimentally

Box 1. Optical tweezers are based on the principle of the conservation of linear momentum, by which a microscopic transparent object (e.g., a polystyrene or silica bead) with an index of refraction higher than that of the surrounding medium deflects an incoming light ray, thus exerting a net force on the object. An optical trap for manipulating single molecules is produced by focusing an infrared beam inside a fluidics chamber, optically trapping a micrometer-sized bead, and measuring either the deflected light using position-sensitive detectors or the bead's position with a CCD camera or back focal plane interferometry. Pulling experiments use dumbbells made of a molecule tethered between two beads (Fig. 1). In single-trap setups, one bead is immobilized in a pipette by air suction, and the other is captured in an optical trap that measures the force exerted on the molecule. By moving the optical trap relative to the pipette we can record the so-called force-distance curve.
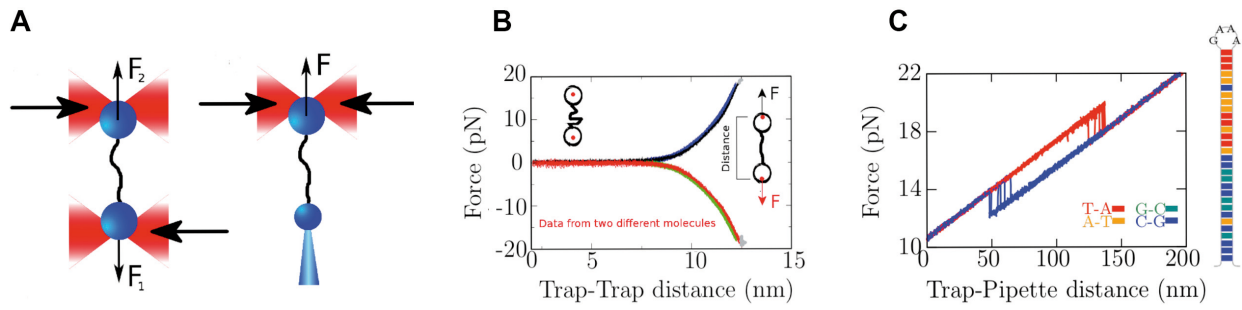

Fig. 1. Schematics of pulling experiments. (**A**) Dual- and single-trap setups. (**B**) Force-distance curves for a DNA molecule pulled in a dual-trap setup. The forces measured at the two traps are of equal magnitude but opposite sign. (**C**) Mechanically unfolding and folding DNA hairpins. The force jumps correspond to unfolding (red) and folding (blue) transitions.

studied over the past several years are the current flowing across a resistance, a colloidal microsphere captured in an optical trap, a biological molecule with two or more conformational states, and a single-electron transistor to cite a few. In general, the extensiveness property of energy is related to the size of the system and to the measurement time; therefore, fluctuations should be difficult to observe in macroscopic systems and over times that are so long that large deviations from the average become exceedingly rare. However, technological developments over the past two decades in the fields of micro- and nanotechnologies and the development of high-temporal resolution cameras, microfluidics devices, photomultipliers, and photodetectors for light detection have enormously expanded our ability to measure these phenomena. Entropy production and energy fluctuation measurements in non-equilibrium systems are now accessible to the experimentalist and our opportunities to expand our knowledge in this exciting field are steadily growing.

Small systems and single-molecule experiments

Biology investigates all aspects of living organisms whereas traditionally physics has largely ignored the study of the mat-

ter of living systems. Yet, except for its complexity, living matter is the same as ordinary matter. It was mainly 19th century chemists who appreciated the importance of understanding living matter, through the newborn discipline of biochemistry. However, concepts such as space, time, force, and energy are not only fundamental quantities in physics they are also central to biology. Thus, the field of biophysics applies the concepts and techniques of physics to study living beings. Physical techniques, such as X-ray diffraction, nuclear magnetic resonance, and electron microscopy, are among those that have contributed to the recent revolution in biology. Conversely, many physicists are now using biological systems as the basis of physical models to test and scrutinize new physical theories.

A prominent example of this trend is the recent developments in single-molecule biophysics, in which individual biological molecules are manipulated one at a time, not only to unravel the most complex biomolecular reactions but also to discover new aspects of biological organization or even to challenge physical theories in statistical mechanics. Biological matter is intrinsically soft, with weak molecular forces (electrostatic, hydrophobic, etc.) responsible for its thermodynamic stability. Moreover, typical energies involved in remodeling processes fall in the k_BT range, at the level of thermal noise (Box 1). This means that living matter is subject to

strong fluctuations due to the comparable magnitudes of weak interacting forces and the Brownian forces present in an aqueous environment. This feature distinguishes biological matter from ordinary matter and makes the former an ideal "playground" to investigate non-equilibrium phenomena.

The possibility of manipulating one molecule at a time offers exciting prospects to acquire valuable information about molecular processes [17]. Atomic force microscopy and magnetic and optical tweezers are commonly employed techniques (Box 1) that enable measurements of the elastic properties of biological polymers, the thermodynamic and kinetic stability of molecular folders (nucleic acid structures, proteins), DNA-protein and DNA-peptide interactions (e.g., intercalation, condensation, aggregation phenomena), protein-protein interactions (e.g., ligand-receptor binding), and molecular motors (e.g., cellular transport, DNA-RNA polymerases, ATPases and proton pumps, viral packaging motors, topoisomerases, helicases). For example, it is nowadays possible to attach a DNA molecule between two beads and, by pulling on its two phosphate strands, to unzip it (Fig. 1). By varying the pulling speed, both the force and the work distributions can be measured, which provides valuable information about the folding process (free energy, kinetic rates, and folding pathways).

Single-molecule biophysics is not restricted to investigations of the most complex biomolecular processes (such as segregation of the DNA chromatids during cellular division to cite a remarkable example), it can also be used to study and characterize the mechanical properties in single cells. For example, micrometer-sized beads can be passively attached to the cellular cytoskeleton via integrin receptors located at the cellular membrane and the power spectrum of the position of the bead then measured using time-resolved fluctuation spectroscopy. This type of measurements provides valuable information about the viscoelastic properties of the cell. This novel type of cellular characterization is called mechanical phenotyping, and explorations using this approach are just beginning.

Fluctuation theorems: where do we stand?

According to the second law of thermodynamics, any irreversible transformation produces entropy and therefore increases the total entropy of the universe. For example, when a spoon is used to gently stir a cup of coffee, the work exerted by the spoon is dissipated in the form of heat. However, as previously explained for the case of electric current flowing across a resistance, this statement only holds on average and is apparent in macroscopic systems or for very long times. In small systems, Brownian forces introduce large fluctuations in measurable quantities, such as work or heat, and actual entropy production values vary across repetitions of the same experiment.

Fluctuation theorems (FTs) quantify the occurrence of negative entropy production events relative to positive ones. Let us suppose that a system in a non-equilibrium state produces or consumes a given amount of entropy S_t along a trajectory or path of time t. In general, FTs obey a simple mathematical relation of the following type [9,10]:

$$\frac{P(S_t)}{P(-S_t)} = e^{\frac{S_t}{k_B}} \qquad (1)$$

with S_t the entropy production during time t, P its probability distribution, and k_B is the Boltzmann constant. This very simple relation tells us two things. First, positive S_t trajectories are exponentially more probable than negative S_t ones, the overall probability of negative S_t trajectories being exponentially suppressed both over time and increasing system size. For a macroscopic system in which the number of degrees of freedom is of the order of Avogadro's number (~10^{23}), the extensiveness property of S_t shows how such negative events are severely penalized with exceedingly small probabilities (on the order of $e^{-10^{23}}$). Second, by rewriting the previous expression as $e^{-S_t/k_B} P(S_t) = P(-S_t)$ and integrating over S_t we get $\langle e^{(-S_t/k_B)} \rangle = 1$ (the probability P is normalized to 1) where <...> denotes an average over many repetitions of the same experiment. A result of this type is often known as the Jarzynski equality; it was first obtained by G. Kochkov and B. Kuzovlev in 1991 and later derived by Chris Jarzynski in 1997 in a different non-equilibrium setting. As we explain below, Jarzynski was also the first to recognize the importance of this equality for free energy calculations, a result with practical implications. A corollary of the Jarzynski equality is that $\langle S_t \rangle \geq 0$, a result that we recognize as the second law of thermodynamics. The beauty of Eq. (1) and the Jarzynski equality lies in the fact that the second law does not appear to be an inequality, but an equality instead.

There are three major categories of non-equilibrium systems: transient systems, steady-state systems, and aging systems. Roughly speaking, a suitable form of entropy production S always exists. In transient systems (initially in thermal equilibrium, but driven out of equilibrium by the action of time-dependent external forces), this quantity corresponds

Box 2. One of the main advantages of transient fluctuation theorems (FTs) is the possibility to determine free energy differences from irreversible work measurements. A long-held postulate of thermodynamics, that the equilibrium free energy difference between two states can only be measured through the work produced in a reversible transformation, is now disputed. The Jarzynski equality allows us to extract free energy differences from irreversible transformations [8]. By repeatedly measuring the irreversible work exerted upon the system, the free energy difference can be extracted by exponentially averaging the work values, $\langle e^{(-W/k_B T)}\rangle = e^{(-\Delta G/k_B T)}$ or $\Delta G = -k_B T log(\langle e^{(-W/k_B T)}\rangle)$. The average must be determined from an infinite number of repetitions of the same experiment, which is unfeasible. Due to the exponential character of the average, the Jarzynski equality is strongly biased for a finite number of experiments N; however, it is possible to extract accurate estimates of ΔG by exploiting the dependence of the free energy estimator on N. An extension of the Jarzynski equality is the FT by Crooks [9] (given by Eq.1 with S_t equal to W_{dis}/T), which allows determination of the free energies of native structures from bi-directional pulling experiments, i.e., by combining unfolding (forward) and folding (reverse) work measurements. A 2005 experiment [10] demonstrated how molecular free energy differences in RNA molecules could be determined from irreversible pulling experiments.
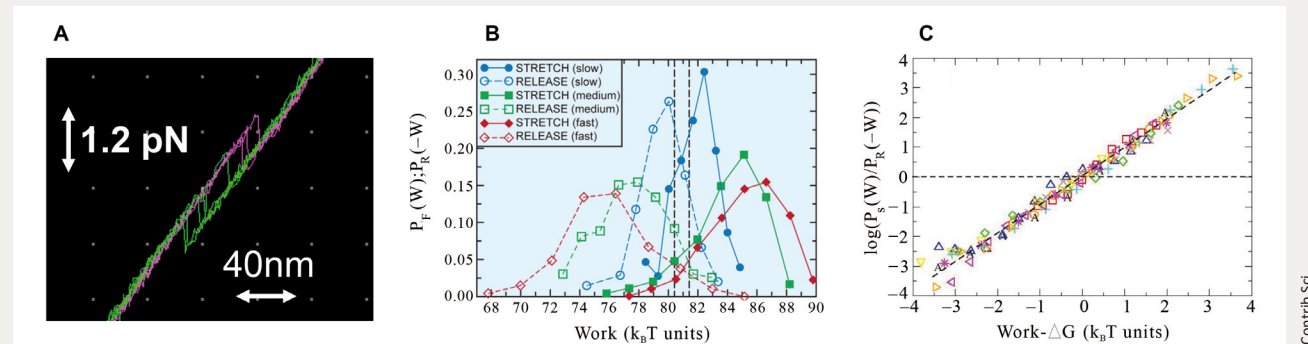

Fig. 2. (**A**) Pulling curves of a 20-basepair DNA hairpin. The molecule unravels around 15 pN. (**B**) Forward and reverse work distributions at three pulling rates: 1 (blue), 5 (green), and 15 (red) pN/s. Distributions cross at Work=DG for all pulling rates. (**C**) Log-normal plot of the ratio between the forward and reverse work distributions.

Different research groups worldwide have applied the technique to extract free energy differences in nucleic acids and proteins, all examples of molecular transformations driven by intramolecular forces (Fig. 2). The domain of applicability of fluctuation theorems has been also extended to extract free energies of kinetic states (i.e., states that are metastable such as intermediate and misfolded states) and intermolecular interactions such as ligand binding reactions [1]. Theoretical studies and experiments show how fluctuation relations are applicable to a wide range of systems in varied conditions: from high to low dissipation, from short to long times or from weakly to strongly interacting systems. In the purely physics domain it has been successfully applied to mesoscopic systems such as beads in optical traps, electric resistances, single electron transistors, Bose-Einstein condensates, etc.... Time will show the overall implications of this fascinating result.

to the amount of dissipated work W_{dis} divided by the temperature T, $S = W_{dis}/T$. On the other hand, W_{dis} equals the total work W exerted by the external forces minus the free energy difference ΔG, $W_{dis} = W - \Delta G$; ΔG also equals the reversible work or the work performed on the system under quasi-static conditions, i.e., in an infinitely slow transformation. In steady-state systems (driven to a stationary state by the action of time-dependent or non-conservative forces, such as the previous example of the electric current flowing through a resistance), the entropy production S equals the work done by the external agent divided by the temperature, $S = W/T$. Finally, in aging systems (relaxational systems that equilibrate over very long-time scales), the heat Q released to the environment during the relaxation process is the key

quantity, $S = Q/T$. Transient FTs have been used to recover free energy differences through the Jarzynski equality $\langle e^{(-S_t/k_B)}\rangle = \langle e^{(-W_{dis}/k_B T)}\rangle = 1$ or $\langle e^{-W/k_B T}\rangle = e^{-\Delta G/k_B T}$, by exponentially averaging the work over many experiments (Box 2). Beyond recovering free energy differences the steady-state FT might be applicable to molecular motors in making inferences about the properties of their mechanochemical cycles (see below).

The experimental measurement of work fluctuations in molecular systems irreversibly pulled by mechanical forces not only has practical advantages, but might also provide new perspectives that contribute to our understanding of living matter. Indeed, one might hypothesize that the marvelous complexity and efficiency of molecular systems in biology

(and biological organisms in general) are the result of an evolutionary process that has taken advantage of such large energy fluctuations in a way that is yet unknown to us. In fact, by rectifying thermal fluctuations, molecular motors have reached astonishing large efficiencies, observable in many enzymatic reactions (such as translocating motors in the cell powered by ATP hydrolysis or energy conversion by light-harvesting complexes in photosynthetic reactions), that have yet to be paralleled by human-designed systems. The importance of the weak forces (hydrogen bonds, electrostatic, hydrophobic) responsible for remodeling events at the molecular and cellular level in low-energy processes at the level of thermal noise suggests that fluctuations and large deviations have played crucial roles during molecular evolution.

From energy to information: thermodynamic inference

In 1867, James Clerk Maxwell, the Scottish scientist who unified electricity and magnetism, proposed a thought experiment to violate the second law of thermodynamics. Maxwell imagined *a very small intelligent being endowed with free will, and fine enough tactile and perceptive organization to give him the faculty of observing and influencing individual molecules of matter* [3]. How a Maxwell demon operates is shown in Fig. 3 (left). In Maxwell's thought experiment, two chambers of a gas kept at equal temperatures are separated by an adiabatic wall with a small hole and a gate that can be opened and closed by the demon. By observing the speed of the individual molecules, the demon selectively opens and closes the gate to separate fast from slow molecules creating a net temperature difference between the two chambers.

The demon can do this effortlessly, without the expenditure of work, thereby violating the second law. There have been several attempts to exorcise the Maxwell demon, but the definitive resolution of the paradox came from the theory of computing. In the 1960s, Rolf Landauer, from IBM, demonstrated that, by recording the information, the Maxwell demon's system never returns to its original state unless information is erased after each observation in a cycle. However, the erasure of information increases the overall entropy, ultimately restoring the validity of the second law. The Maxwell demon can be experimentally realized in the Szilard engine (Fig. 3, right). A demon observes the position of a single particle in a gas chamber in contact with a thermal bath at temperature *T*. When the particle occupies one of the halves of the chamber, a movable wall and a pulley mecha-

nism capable of pulling a weight are implemented in the middle of the chamber. The molecule then pushes against the wall, lifting up the weight. When the full volume of the chamber is finally restored, a cycle has been completed and the process starts anew. Along each cycle heat is transferred from the bath to the system and fully converted into work, violating the second law. The maximum amount of work that can be extracted per cycle, $W_{\max}$, equals the net heat transferred from the bath, $W_{\max} = Q = T\Delta S = k_B T \log(2)$. This value of $W_{\max}$ equals the maximum work that can be extracted by a single-bit Szilard engine in the classical regime, often called the Landauer limit.

The Szilard engine, as an example of a Maxwell demon, can be experimentally realized in small systems such as quantum systems, electronic devices and, more recently, in single molecules. As expected in small systems, there are large fluctuations in the amount of work that can be extracted along a cycle. In the convention that work W extracted from the system is negative (whereas delivered work is positive) this amounts to saying that $W \geq -W_{\max} = -k_B T \log(2)$, or that the average extracted work per cycle cannot exceed the Landauer limit.

Alongside these developments, FTs have now been generally extended to the case in which there is information feedback. A system is driven out of equilibrium by the action of an external agent; however, the non-equilibrium protocol is changed depending on the outcome of one (or more) measurements taken at specific times (discrete time feedback) or when a continuously monitored observable fulfills a specific condition (continuous time feedback). In this situation, the Jarzynski equality $\langle e^{(-W_{dis}/k_B T)} \rangle = 1$ becomes $\langle e^{[-(W_{dis}-I/k_B T)]} \rangle = 1$, where *I* is a new quantity called information that is directly related to the feedback protocol [13]. The convex property of the exponential function immediately leads to $\langle W_{dis} \rangle \geq -k_B T \langle I \rangle$ or $\langle W \rangle \geq \Delta G - k_B T \langle I \rangle$, meaning that the minimum amount of work exerted during a transformation in the presence of feedback can be less than ΔG. For cyclic protocols where $\Delta G = 0$, feedback enables the extraction, on average, of a maximum amount of (negative) work equal to $\langle W \rangle_{\max} \geq -k_B T \langle I \rangle$, with $\langle I \rangle \geq 0$. The mathematical expression for the path-dependent information *I* is in general complicated and depends on the specific feedback protocol. However, for the simple one-bit Szilard engine case one has $\langle I \rangle = \log(2)$.

Information-to-energy conversion experiments show that energy and information are highly related quantities, one does not proceed without the other. Another twist in this exciting field is provided by thermodynamic inference, or the possibility to extract useful information about a non-equilib-
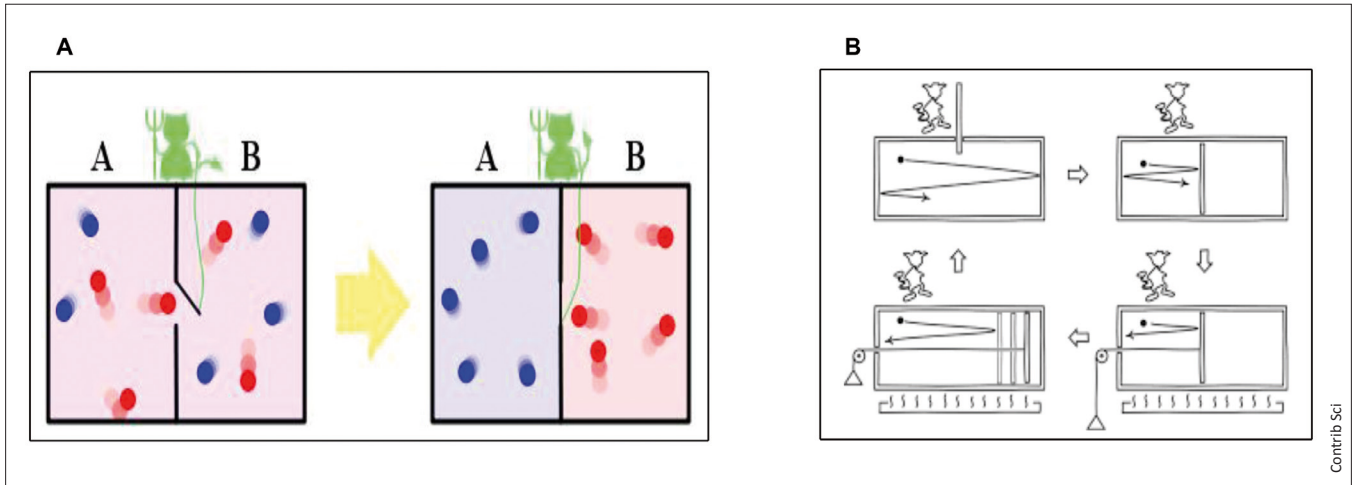
Fig. 3. (A) Maxwell demon. A small being (green) generates a temperature gradient without the expenditure of work. (B) Szilard engine. The same small being fully converts heat into work without any further change.

rium system by imposing the validity of the FT [16]. Let us consider a DNA molecule tethered between two beads in a dual-trap optical tweezers setup. Dual traps are useful for the experimentalist because of their high resolution and reduced instrumental drift. The setup can be used to repeatedly pull a molecule along a cycle by moving one optical trap while the other remains at rest (Fig. 4). The bead captured in the moving trap is then dragged through water, where it is subject to Stokes friction. The total work W exerted upon the system as measured by the force recorded in the moving trap has two contributions: the work exerted to stretch the DNA molecule and the work required to move the bead in the fluid against frictional forces. The total dissipated work D along a cycle satisfies Crooks' FT [Eq. (1)], with $S = D/T$. However, some dual-trap setups cannot measure the force in the moving trap but only in the trap that remains at rest during the pulling experiment. As the bead in the trap at rest hardly moves along the pulling cycle, the Stokes friction force experienced by the bead is smaller in that trap than in the moving trap (cyan arrows in Fig. 4).

The dissipated work D' extracted from the force measured in the trap at rest is missing a dissipative component and does not satisfy the FT of Eq. (1). We might say that devices measuring the force in the trap at rest ignore an essential part of the total work (accounted for in the moving trap instead), resulting in a partial work measurement. In other words, these devices cannot be used to measure full entropy production in a non-equilibrium experiment or to test the validity of FTs. However, one can impose the validity of the FT to infer, from only partial work measurements in the trap at rest, the full work distribution one would measure in the moving trap. In the case of the DNA molecule shown in Fig. 4, this is accomplished by shifting the partial work distribution $P(D')$ by a constant value Δ, which is equal to the average dissipation of the center of mass in the dumbbell along a cycle. We call this thermodynamic inference: it is the procedure by which we infer the full entropy production distribution in a non-equilibrium experiment from partial work (D') measurements.

Thermodynamic inference is therefore a powerful concept that is applicable whenever full entropy production in a non-equilibrium system is not measurable, either because it cannot be experimentally accessed or because it has unexpected hidden contributions. It might be used in a wide range of situations, such as extracting the free energies of (hidden) kinetic states in single molecules or in obtaining useful information about the mechanochemical cycle in ATP-powered machines. It might be also used to quantify randomness in heterogeneous molecular ensembles, such as protein sequences exhibiting a multiplicity of native states or sequence ensembles of nucleic acids and proteins generated in molecular evolution experiments. Finally we stress the importance of measuring work fluctuations in gauging the power of the thermodynamic inference approach. The sole measurement of the average partial work precludes inference of the average total work, as it is not possible to impose the validity of the FT using only measured average values. In this regard, thermodynamic inference is a key feature of small systems and is without parallel in irreversible thermodynamics of macroscopic systems.
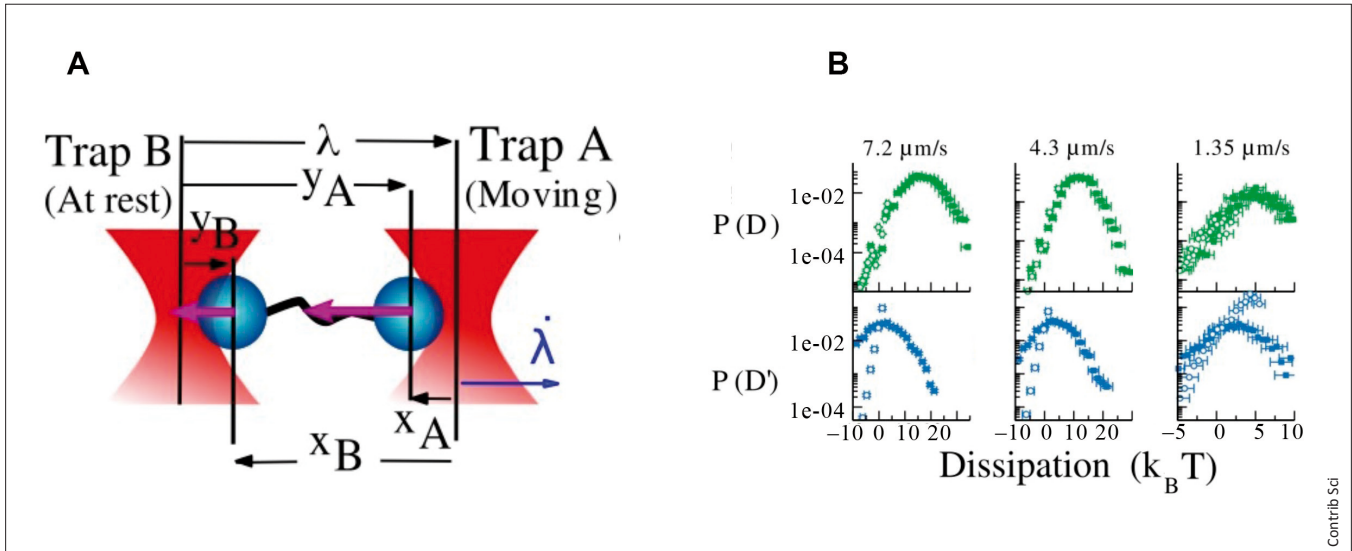
Fig. 4. Thermodynamic inference. (**A**) Schematics of a dual trap with moving trap A at speed λ° and trap B at rest in the water frame. Cyan arrows indicate drag forces on each bead. The energy must be defined in terms of the configurational variables yA,yB with respect to the frame at rest rather than the moving-frame variables xA,xB. (**B**) Dissipated work D,D' distributions measured for forces recorded in traps A and B respectively when pulling a DNA molecule at different speeds along a cycle. Filled symbols are the $P(x)$ and open symbols are $P(-x)e^{(x/k_BT)}$ with x = D(green), D'(blue). Only D satisfies the FT Eq.(1).

Lessons from glassy systems: the effective temperature

The validity of Eq. (1) relies on properly accounting for the full entropy production S_t. However, as previously explained, sometimes only a partial entropy production measurement S_t' is feasible. With the use of several simplifying assumptions [2] one can prove that S_t' satisfies a modified version of the FT shown in Eq. (2):

$$\frac{P(S_t')}{P(-S_t')} = e^{x\frac{S_t'}{k_B}} \qquad (2)$$

with x a dimensionless quantity. Equation (2) is often called an x-FT and it differs from Eq. (1) only by the pre-factor x in the exponent. In general, the x-FT is not an exact result, but it does hold in the Gaussian regime exemplified by the above-described DNA molecule pulled in a dual-trap setup, where $\langle S't\rangle = x\langle S_t\rangle$. The inequality $\langle S't\rangle \leq \langle S_t\rangle$ then leads to x < 1. What is the physical interpretation of x? According to the previous relation, x is the fraction of the total entropy production measured in the trap at rest. In other words, if x is < 1, then a part of the total entropy production is missing in the measurement.

A very interesting connection has recently emerged in the context of glassy systems. Glasses and spin glasses are systems containing a high degree of structural disorder. They

relax to equilibrium extremely slowly after a quench (i.e., a very fast change in external parameters, such as temperature and volume). This slow relaxation implies an extremely low entropy production rate. In many respects the amount of dissipation falls within the small systems regime, where fluctuations and large deviations from the average behavior are characteristic features. Interestingly, however, it has been demonstrated that glassy systems fulfill an x-FT of the type shown in Eq. (2), with x being equal to the so-called fluctuation-dissipation ratio describing violations of the fluctuation-dissipation theorem that have been interpreted with the notion of an effective temperature [6]. Roughly speaking, the effective temperature T_{eff} quantifies how fast correlations decay in the glassy state vs. in a system thermally equilibrated at the quenching temperature. Because of the large viscosity of supercooled glasses (in may change by twenty orders of magnitude in a narrow temperature range), values of T_{eff} are often too high (thousands of Kelvins in many cases). What is the physical significance of these strikingly high temperatures? For glassy systems characterized by a single relaxational timescale one finds $x = T/T_{eff}$; therefore the value of x in glasses is expected to be small. We hypothesize that the physical meaning of x and T_{eff} should not be considered from an energetic viewpoint but rather from an informational one. The low values of x (and the correspondingly high values of T_{eff}) should be interpreted in terms of missing information in

the aging state. In other words, the average entropy production commonly measured in aging experiments $\langle S'_t \rangle$ is just a tiny fraction (x) of the total entropy produced $\langle S_t \rangle$. An informational theoretical interpretation of the fluctuation-dissipation ratio should also be possible in other non-equilibrium contexts, e.g., in non-equilibrium steady states that violate the fluctuation dissipation theorem and show the emergence of effective temperatures [8].

The role of information in biology

In 1944, Erwin Schrödinger published an enlightening monograph titled "What is life?", in which he wrote [20]: *The large and important and very much discussed question is: How can the events in space and time which take place within the spatial boundary of a living organism be accounted for by physics and chemistry?* We accept that living beings do not violate fundamental laws of physics. However, we also immediately recognize that living beings are very special. They seem to circumvent or mock the laws of physics as we understand them: a stone will fall because it is acted upon by gravity, whereas birds fly whenever they feel the need to do so. Biologists refer to this behavior as teleonomy, which recognizes that living beings have their own agenda; that is, they move, jump, play, eat, reproduce, plan, shop, do business, carry out research, etc. [15]. Unlike ordinary matter, living beings are always part of a population and an ecological niche. The physicist might call this an ensemble of individuals. Biological populations evolve under the rules of Darwinian selection, in which those individuals that best respond to the pressures of their environment succeed over those less well able to do so. Darwinian evolution rests on a dynamics of a very special kind, in which mutations and selective amplifications of the fittest species determine the evolving phenotypes. In the eyes of a physicist, evolving populations produce a startling non-stationary state in which basic thermodynamics concepts such as energy, matter, entropy, and information are intertwined in a complex and undecipherable manner [11].

Living matter has two features: it is heterogeneous and soft. Because cell populations are intrinsically heterogeneous, experiments that aim to reveal their features are difficult to reproduce: the same strain, the same environmental conditions, etc., often produces different outcomes. Heterogeneity is not only restricted to cells, it is also present at the molecular level. Myoglobin, the oxygen carrier protein in the muscle tissue of vertebrates, is known to fold into a heterogeneous set of different native structures, all them able to bind oxygen. Other proteins (e.g., enzymes and polyclonal antibodies in the immune system) are also able to assume a multiplicity of native states. In addition, living matter is soft and actively subjected to remodeling. Embedded in noisy aqueous environments, the forces inside biological structures must be strong enough to keep them stable and, at the same time, weak enough for the structures to continuously remodel in response to changing environments. It is not by chance that the fundamental energy scale of statistical physicists equals that of biochemists (1 $k_B T$ = 0.6kcal/mol at 298K). In fact, fundamental biological forces operate at the edge of the thermal noise level and the stabilizing free energies of macromolecules in tissues are on the order of a few kcal/mol or $k_B T$. As enthalpy (H) and entropy (TS) contributions are typically much higher, such low free energies can be achieved only by a fine compensation between both contributions. Molecular pathways in the cell have been finely tuned to operate within a narrow range of conditions, and they are extremely sensitive to small variations in the environment. The task of precisely determining the energies involved in molecular pathways is a daunting one, due to the many endogenous and exogenous factors that are beyond control, molecular heterogeneity being a relevant one. In this setting, inference may offer a powerful approach to deepening our knowledge of biological systems. The enormous amount of information needed to describe biological systems and the sensitivity of these systems to various noise sources and heterogeneous disorder may favor inference reasoning over deductive knowledge. Inference represents knowledge that is not accessible by direct measurement; it is knowledge consistently implied by the validity of physical laws. Whether inference is the correct tool with which to face the new challenges in biology remains to be seen.

Closing remarks

Energy, entropy, and information are the three main driving forces underlying the remodeling of biological matter. While thermodynamic processes in ordinary matter are driven by free-energy minimization (i.e., competition between energy and entropy), living matter seems to be predominantly governed by information flows across many different organizational and stratification levels, leading to complex integrated biological cells and organisms and resulting in what has been dubbed as "molecular vitalism" [14]. How the non-equilibrium physics of small systems can contribute to furthering our understanding of the marvelous attributes of living matter

remains to be determined. Nonetheless, the experiments and theories developed in statistical physics over the past decades have demonstrated the prominent role of information, a quantity that physicists generally identify with entropy but which may be a more general one when used to explain the emergent complexity of biological matter. One of the most appealing features of the Jarzynski equality and fluctuation relations is that they allow us to recover the second law of thermodynamics as a particular case of a more general mathematical equality. This raises the intriguing question whether the second law is ultimately a conservation law rather than an inequality, paralleling the mathematical equality represented by the first law of thermodynamics. From our perspective statistical mechanics and biophysics are intimately related disciplines; in the latter, biological systems are used to investigate non-equilibrium phenomena and, perhaps, to uncover new physical laws as well. The non-equilibrium physics of small systems might thereby represent the first step in finally unraveling new secrets of nature. ◪

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References

1. Alemany A, Mossa A, Junier I, Ritort F (2012) Experimental free-energy measurements of kinetic molecular states using fluctuation theorems. Nature Physics 8:688-694 doi:10.1038/nphys2375
2. Alemany A, Ribezzi-Crivellari M, Ritort F (2015) From free energy measurements to thermodynamic inference in non-equilibrium small systems. New J. Phys. 17:075009
3. Bennett CH (1987) Demons, engines and the second law. Scientific American 257:108-116
4. Bustamante C, Liphardt J, Ritort F (2005) The non-equilibrium thermodynamics of small systems. Phys. Today 58:43-48
5. Collin D, Ritort F, Jarzynski C, Smith SB, Tinoco I, Bustamante C (2005) Verification of the Crooks fluctuation theorem and recovery of RNA folding free energies. Nature 437:231-234 doi:10.1038/nature04061
6. Crisanti A, Picco M, Ritort F (2013) Fluctuation relation for weakly ergodic aging systems. Phys. Rev. Lett. 110:080601 doi:10.1103/PhysRevLett.110.080601
7. Crooks GE (1999) Entropy production fluctuation theorem and the non-equilibrium work relation for free-energy differences. Phys. Rev. E 60:2721-2726
8. Dieterich E, Camunas-Soler J, Ribezzi-Crivellari M, Seifert M, Ritort F (2015) Single-molecule measurement of the effective temperature in non-equilibrium steady states. Nat. Phys. 11:971-977
9. Evans DJ, Searles D (2002) The fluctuation theorem. Adv. Phys. 51:1529-1585
10. Gallavotti G, Cohen EGD (1995) Dynamical ensembles in non-equilibrium statistical mechanics. Phys. Rev. Lett. 74:2694-2697 doi:10.1103/PhysRevLett.74.2694
11. Goldenfeld N, Woese C (2011) Life is Physics. Annu Rev Condens. Matter Phys. 2:375-399
12. Jarzynski C (1997) Non-equilibrium equality for free-energy differences. Phys Rev Lett 78: 2690 doi:10.1103/PhysRevLett.78.2690
13. Jarzynski C (2011) Equalities and inequalities: Irreversibility and the second law of thermodynamics at the nanoscale. Ann Rev Condens Matter Phys. 2:329-351 doi:10.1146/annurev-conmatphys-062910-140506
14. Kirschner M, Gerhart J, Mitchison T (2000) Molecular "vitalism". Cell 100:79-88 doi:10.1016/S0092-8674(00)81685-2
15. Pross A (2012) What is life? Oxford University Press
16. Ribezzi-Crivellari M, Ritort F (2014) Free-energy inference from partial work measurements in small systems. Proc Natl Acad Sci 111:E3386-3394 doi:10.1073/pnas.1320006111
17. Ritort F (2006) Single molecule experiments in biological physics: methods and applications. Journal of Physics C (Condensed Matter) 18: R531-R583 doi:10.1088/0953-8984/18/32/R01
18. Ritort F (2008) Non-equilibrium fluctuations in small systems: from physics to biology. Adv Chem Phys 137:31-123
19. Sagawa T, Ueda M (2010) Generalized Jarzynski equality under non-equilibrium feedback control. Phys.Rev.Lett. 104:090602 doi:10.1103/PhysRevLett.104.090602
20. Schrödinger E (1944) What is life? Cambridge University Press
21. Seifert U (2012) Stochastic thermodynamics, fluctuation theorems and molecular machines. Rep Prog Phys 75:126001 doi:10.1088/0034-4885/75/12/126001

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