In retrospect

25 years of nanoscale thermodynamics

Chase P. Broedersz & Pierre Ronceray

A paper published in 1997 brought the thermodynamics of the nineteenth century into the twenty-first century – expanding the physics of transformations involved in the operation of steam engines to the realm of molecular motors.

The history of thermodynamics begins in 1824, when the French engineer Nicolas Léonard Sadi Carnot observed that a steam engine's efficiency has a natural limit. The German physicist Rudolf Clausius made sense of this with a mathematical inequality that became known as the second law of thermodynamics: a quantity that he called entropy keeps increasing until a system reaches equilibrium. This law has since become a fixed feature of university physics curricula, and remained unimproved for more than a century – until 25 years ago, when Christopher Jarzynski[1] proposed an improved relationship that has intriguing consequences for microscopic systems, such as the tiny engines that drive living organisms.

To understand the second law of thermodynamics, imagine stretching a rubber band. This is an example of a thermodynamic transformation – a process by which a system moves from one state of thermodynamic equilibrium to another. To stretch the band, you have to perform work and use up some energy. Part of this work is stored, and remains thermodynamically accessible. This is known as the free energy and can be retrieved by relaxing the band. The rest of the work is eventually irreversibly released as heat into the system's surroundings – and the faster you stretch the band, the more work will be dissipated.

The way that the second law describes the physics of this process is best understood in terms of the Austrian physicist Ludwig Boltzmann's interpretation of entropy as a measure of randomness. The second law states that the work that is dissipated into the system's surroundings is greater than or equal to zero on average: the dissipated work warms up the environment, thus increasing its randomness – and its entropy. Unfortunately, there's no way of reliably extracting heat from the environment by stretching and relaxing the rubber band.

In just four pages, Jarzynski used mathematical tools that had been around since Boltzmann's time to come up with a version of the second law that is just as general as the original, but is couched as an equality rather than an inequality. It states that an exponential function of the work that you put in, averaged over many experiments, is exactly equal to the same function of the free-energy change. This implies that when the exponential of negative dissipated work is averaged over many experiments, the result is exactly equal to one. This mathematical expression is known as the Jarzynski equality, and it puts tighter constraints on thermodynamic transformations than does the second law. In fact, the second law follows directly from Jarzynski's equality (Fig. 1).

So, let's imagine repeating our stretching experiment: the amount of work dissipated will vary each time the experiment is performed, because the environment is subject to random thermal variations. Whereas the second law deals only with the average behaviour of the work dissipated by a certain transformation, Jarzynski's equality also encompasses these fluctuations. For a macroscopic system such as a rubber band, the fluctuations are negligible, but they become crucial for microscopic systems such as single biomolecules. Indeed, thermal randomness has the largest impact on nanoscale systems operating close to the thermal energy associated with these fluctuations, which is a very small 4×10^{-21} joules at room temperature. The main consequences of Jarzynski's equality thus lie in the microscopic realm, where randomness has a prominent role.

At such small scales, fluctuations are so large that some events actually extract heat from the environment – allowing a biomolecule to be stretched with less work than the change in the system's free energy. On average, though, the second law still stands: these rare events can't be used to produce energy for free. This is similar to a lottery, in which it is possible on rare occasions to earn money, even though players lose money on average. In Jarzynski's equality, these rare events have a prominent role, because their importance is amplified by the exponential function (Fig. 1). The equality reveals a subtle balance between these rare lottery-winning events that extract energy from the thermal environment, and the typical losers that dissipate work.

To test this theoretical prediction, a thermodynamic experiment was set up at this microscopic scale. Five years after Jarzynski's

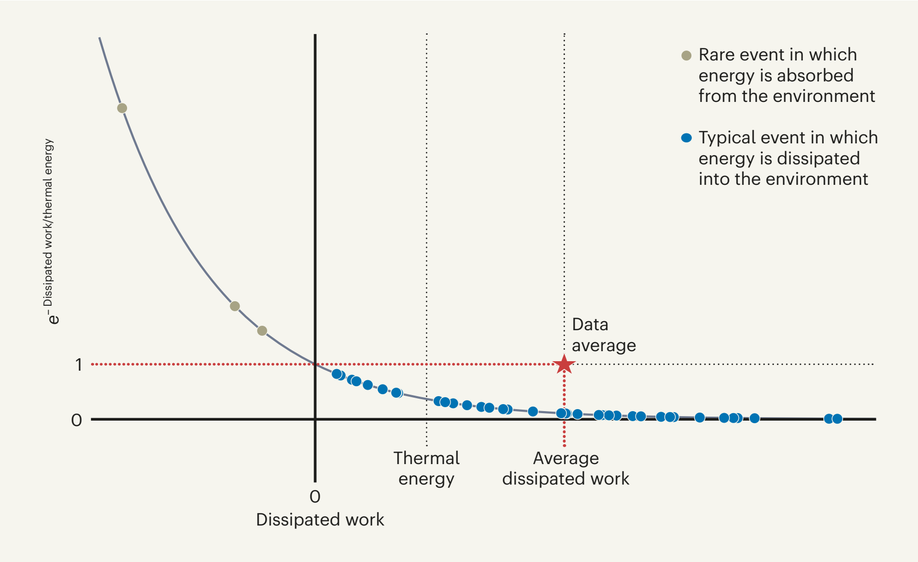

Figure 1 | Improving the second law of thermodynamics. When a system is moved from one state of thermodynamic equilibrium to another – for example, when a single biomolecule is stretched – a certain amount of the work exerted is dissipated and lost into the surroundings. If this transformation is repeated many times, the work that is dissipated will vary from one experiment to the next. Most of the time, it is positive, but in a few, rare events, the system actually absorbs energy from the environment. Jarzynski[1] derived a mathematical equality that implies that the average of the exponential of negative dissipated work, relative to the thermal energy, is exactly one. Because the exponential function curves upwards, the average dissipated work is always greater than or equal to zero, which is the second law of thermodynamics.

discovery, a team of biophysicists used light traps called optical tweezers to manipulate a single molecule of RNA, which interacts with itself to form structures resembling hairpins. By repeatedly opening and closing an RNA hairpin, the researchers showed that the work fluctuations accurately obeyed the Jarzynski relation[2].

The nanometre scale is also the realm of molecular motors: protein complexes that act as mechanical engines in the cell. Molecular motors can perform work by stretching biopolymers or by transporting cargo against a drag force. In doing so, they drive mechanical processes — from cell division to muscle contraction — occurring at larger scales. A single molecular motor is fuelled by molecules that each provide around 20 times the thermal energy at room temperature. This means that the operation of biomolecular engines is, in principle, constrained by Jarzynski's equality, making his research the first step in revealing how the nanoscale thermodynamics of molecular motors[3] goes beyond the macroscopic thermodynamics of steam engines.

Jarzynski's discovery — an equality hidden in plain sight behind the second law's inequality — also had a major impact on the physics of non-equilibrium processes. Strikingly, the relationship makes no assumptions about how fast a thermodynamic transformation takes place. It thus applies, for example, if the system is driven far from thermal equilibrium during the experiment. This in turn implies that the work fluctuations of a non-equilibrium process are directly related to equilibrium free-energy differences — a surprising result, suggesting that such processes obey general laws that can be derived exactly.

Such laws are known as fluctuation theorems, and they had already started to appear in the years before Jarzynski's result, but their impact had been appreciated by only a narrow group of researchers[4,5]. The most popular fluctuation theorem, Gavin Crooks' refinement of Jarzynski's equality, offers a statistical description of the arrow of time of a process — a way of calculating the probability that a video of an experiment is running forwards or backwards[6]. This surge of fundamental discoveries sparked the development of modern stochastic thermodynamics, which is a mathematical framework describing the thermodynamic properties of microscopic systems ranging from nanometre-scale molecular motors to micrometre-scale colloids[7].

Although Jarzynski's equality remains essential to our understanding of modern statistical physics and has been verified in many contexts[2,8], its direct application has so far proven underwhelming, and it has yet to result in any major technological advances. For instance, although it can be used as a tool to determine free-energy differences in molecular dynamics simulations[9], this application is limited by the fact that the relationship relies on very rare events. A promising class of thermodynamical uncertainty relationship has emerged to describe non-equilibrium fluctuations, which are more practical because they do not rely on such rare events[10,11].

All in all, Jarzynski's article remains a physicist's favourite, and a must-read for students, for its simplicity and elegance, as well as its impact on our understanding of thermodynamics. Rarely has a breakthrough discovery been so clearly evident at the time of its publication.

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Molecular biology

Accessible method maps mutations to biophysics

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A general method that quantifies and disentangles the effects of a gene's mutations on the traits of its protein enables assessments of mutational effects on protein biophysics for many of the proteins of a living organism. **See p.175**

Proteins function through their interactions with other biomolecules, and can be modulated not only by changes to the amino-acid residues at the protein–biomolecule interface, but also by changes at distal sites — a phenomenon called allostery. Discovering which protein sites are allosteric has been difficult, because methods that simply and comprehensively quantify allosteric effects have been lacking. On page 175, Faure *et al.*[1] report the first integrated method to globally map, quantify and distinguish the effects of mutations on allostery and protein stability. Their approach is also easy to do, making it as accessible to geneticists as it is to biophysicists. This method will have broad applications in biotechnology and drug discovery.

Advances in experimental technologies, especially DNA sequencing, have enabled the simultaneous measurement of the effects of thousands of mutations on a single protein[2]. Many hundreds of these high-throughput experiments have been published[3], focusing on assays that can infer mutational effects on a wide range of protein properties and processes — including heat stability, catalytic activity, binding to other proteins and small molecules, cell growth and drug resistance. The COVID-19 pandemic has showcased the value of these technologies, which have been used to screen how mutations in the spike protein of the SARS-CoV-2 virus affect the protein's expression and binding to host receptors[4] and antibodies[5]. Computational methods have also matured, and can now make surprisingly accurate predictions of mutational effects[6] across thousands of proteins without using experimental data[7]. All of these successes have led to the emergence of an international consortium called the Atlas of Variant Effects Alliance (www.varianteffect.org), whose goals include the open sharing of experimental and computational methods among scientists.

However, in practice, neither the experimental nor computational approaches have been able to distinguish the effects of mutations on different protein traits. To predict and, ultimately, engineer proteins that have desired traits requires an understanding of how biophysical properties are encoded by protein sequences.

Furthermore, ambiguities in discerning