SINGLE-MOLECULE EXPERIMENTS

Out of equilibrium

Equilibrium free-energy landscapes supply important information about complex molecules such as nucleic acids and proteins. But can equilibrium landscapes be calculated from measurements on a non-equilibrium system?

Christopher Jarzynski

Single-molecule experiments provide wonderfully direct access to the dynamics of individual molecules and molecular complexes. Tethering a lone strand of DNA (for instance) between a pair of micron-size polystyrene beads and using laser tweezers to haul the beads around allows experimentalists to watch in detail how the nucleic acid responds. They can monitor the distance between the two beads — an indication of the molecule's end-to-end extension — as well as the pulling force exerted by the tweezers. Both the extension and the force fluctuate with time as the molecule and beads are jostled by their thermal surroundings. The game in such experiments is then to convert these fluctuating signals into knowledge about the molecule's behaviour and properties. These include thermodynamic properties such as free-energy profiles that quantify the molecule's propensity to fold into various three-dimensional structures.

This is where statistical physics enters the picture. If the system is maintained in equilibrium by holding the laser tweezers at a fixed pulling force, then constructing a free-energy profile amounts to taking the natural logarithm of a histogram: $G(q) = -k_B T \ln(P(q))$, where k_B is Boltzmann's constant, T denotes temperature, and the distribution $P(q)$ describes the observed equilibrium fluctuations of the molecule's extension, $q(t)$. A potential drawback of this approach is that free-energy barriers are difficult to sample with good statistics: a local maximum in the free-energy profile implies a local minimum in the equilibrium fluctuations. Reporting in *Nature Physics*, Amar Nath Gupta and co-workers[1] validate an alternative strategy for obtaining equilibrium free-energy profiles, which draws on recent progress in non-equilibrium statistical physics.

The strategy involves stretching a single molecule — it could be a nucleic acid[1,2], a protein[3] or even a synthetic supramolecular complex[4] — causing it to evolve from one structure to another, typically from a folded to an unfolded state. This process is generally accompanied by the irreversible dissipation of energy. In 2001, Hummer and Szabo[5] proposed that the data from many repetitions of such a non-equilibrium process can be combined to reconstruct the equilibrium free-energy profile $G(q)$.

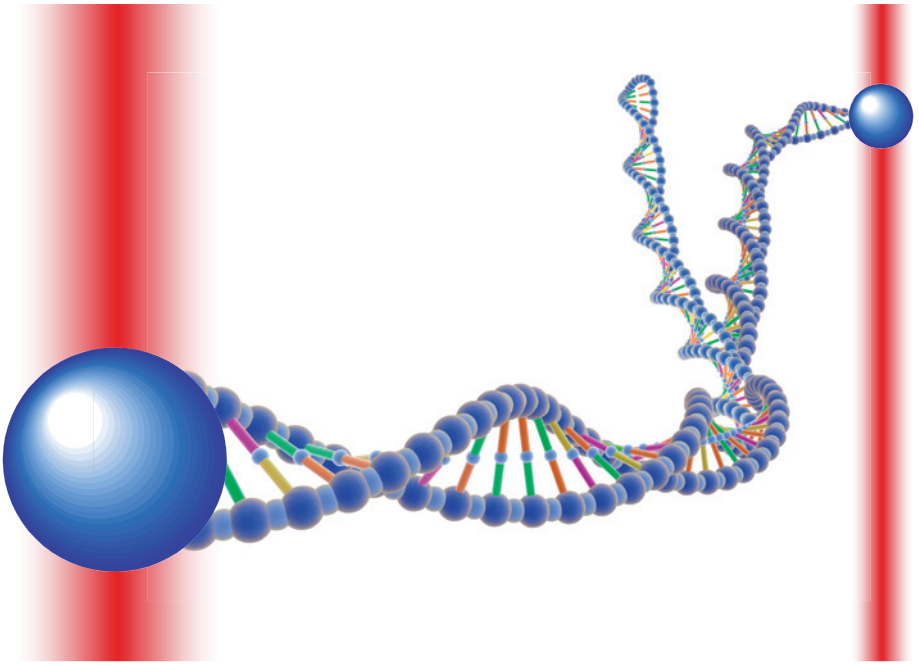

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Figure 1 | DNA at a stretch. Measurements of the fluctuating extension of molecules as force is applied by optical tweezers can be used to reconstruct equilibrium free-energy profiles.

The idea that a system's equilibrium properties can be determined by observing its out-of-equilibrium behaviour stands in contrast to traditional notions of thermodynamics. Although Hummer and Szabo's method had been previously applied to experiments that involve the stretching of single molecules, the method had not been validated by direct comparison with the equilibrium, log-of-a-histogram approach. To this end, Gupta *et al.* performed experiments using two different DNA 'hairpins', designated A and B. These are relatively short pieces of single-stranded DNA that adopt a structure in which the strand folds back on itself. Each end of the hairpin was attached to a DNA 'handle', a longer length of double-stranded DNA, and the two handles were in turn fastened to polystyrene beads, with the entire complex forming a kind of floppy dumb-bell (Fig. 1). A pair of optical traps, or laser tweezers, were then used to grab and manipulate the two beads.

In earlier experiments[6], these DNA hairpins were held at a fixed pulling force, calibrated so that the molecule was equally happy in either the folded (hairpin) or the unfolded structure. By observing the conflicted molecule's excursions back and forth between the two structures, the histogram of equilibrium fluctuations and ultimately the free-energy landscape were obtained. By contrast, in the present experiments, each hairpin was actively unfolded by moving the optical traps apart at a constant speed. In the force-

extension curves obtained from such an experiment, the unfolding event appears as a signature single sawtooth, reflecting the sudden increase in extension and decrease in tension that occurs at the moment of unfolding.

After recording more than 1,500 force-extension curves for hairpin A at various pulling speeds, and nearly 3,000 for hairpin B, Gupta *et al.* analysed their non-equilibrium data using the method proposed by Hummer and Szabo[5]. The resulting free-energy profiles nicely match those obtained from the earlier equilibrium method, providing direct validation of the non-equilibrium approach.

An important issue that arises with both methods is that the extension and force signals reflect not just the unfolding dynamics of the hairpin, but also the noisy fluctuations of the long DNA handles, which blur the free-energy profile. Here, deconvolution techniques, of the sort applied in signal analysis and image processing, can be used to tease out the profile of the hairpin itself. Interestingly, Hummer and Szabo have recently revisited the problem of extracting equilibrium free-energy landscapes from non-equilibrium single-molecule experiments, and have proposed an alternative method based directly on deconvolution[7].

Gupta and colleagues also report measurements on a riboswitch aptamer, a short strand of RNA that adopts partially folded intermediate structures along the way from the folded to the unfolded state. As with the DNA hairpins, the landscape reconstructed using the non-equilibrium approach agrees with that obtained from equilibrium data. However, neither profile resolves the intermediate structures. It is not clear whether this reflects the inherent difficulties of reconstructing a free-energy landscape of sufficiently high resolution, or whether, simply, the extension is not the optimal reaction coordinate for revealing the details of the intermediate states.

Although these experiments rely on observations of the extension and the force, it will be interesting to see what new observables can be added to the single-molecule toolkit as the techniques continue to advance. It will no doubt be equally interesting to follow the theoretical work that incorporates these new data signals into the analysis of single-molecule experiments. ❐

Christopher Jarzynski is at the Department of Chemistry and Biochemistry and the Institute for Physical Science and Technology, University of Maryland, College Park, Maryland 20742, USA.
e-mail: cjarzyns@umd.edu

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PLANETARY SCIENCE

The Trojan is out there

In 1772, Joseph-Louis Lagrange calculated that a small body could be trapped in the orbit of a larger one, if it were positioned either 60° ahead of or 60° behind the larger object. Those positions, now bearing the symbols L_4 and L_5, are two of the series of five so-called Lagrange points in space (the others were defined earlier by Leonhard Euler). At a Lagrange point, any small body — say, a satellite — if subject only to gravity is stationary with respect to two larger bodies — such as a planet and the Sun.

It wasn't until 1906 that the first object trapped at L_4 or L_5 was found: Max Wolf discovered 588 Achilles, an asteroid at L_4 in the Sun–Jupiter system. And only now — M. Connors *et al. Nature* **475,** 481–483 (2011) — has Earth been shown to have a similar 'Trojan' asteroid of its own.

Following Wolf's discovery, more asteroids were found at both L_4 and L_5 around Jupiter, and they are now more than four thousand in number. By convention, and hence the origin of the term 'Trojan asteroid', each is named after a hero of the Trojan War from Greek mythology (a scene of which appears on the Greek vase, pictured); heroes of the Greek camp are clustered at L_4 (Agamemnon, Odysseus, Ajax, Menelaus) and those of Troy at L_5 (Priamus, Troilus, and so on) — although two early discoveries, the Greek Patroclus and Trojan Hektor, were in fact assigned to the wrong camps.

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Trojan asteroids have since been identified at Mars and Neptune. Two of Saturn's moons, Tethys and Dione, have Trojan moons. But no Trojans had ever been seen in Earth's orbit: as seen from Earth, they would dwell in the daylight sky, making detection difficult. However, in data from NASA's Wide-field Infrared Survey Explorer (WISE), backed up by further ground-based observations, Martin Connors and colleagues have found an object that fits the bill.

The asteroid, somewhat less romantically named as 2010 TK_7, is several hundred metres in diameter and is librating about L_4, 60° ahead of Earth in its orbit. Its motion is typical of a Trojan asteroid, and its orbit stable over at least 10,000 years. However, the chaos of its motion is such that attempts to map its motion far into the future or the past are not accurate. Connors *et al.* have run simulations with varying parameters to investigate the possibilities — including that of 'jumping' to another Lagrange point, as the Jupiter Trojan 1868 Thersites is thought to have done. More will be learned through further observations, but its dynamics are such that the origin and ultimate fate of 2010 TK_7 will probably never be known.

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