ENERGY IN MOTION

How the nanomachines of life harvest randomness to do the cells' work

BY DAVIDE CASTELVECCHI

Occasionally, scientists stumble upon what seems to be a free lunch. But they're not concerned about possibly violating the laws of economics. It would be much more shocking to break the laws of physics.

To physicists, the no-free-lunch rule is precious. One form of it is the first law of thermodynamics, which says that energy cannot be created from nothing. The second law of thermodynamics goes even further, declaring not only that lunches are never free but also that they come at some minimum price.

Nonetheless, some natural phenomena seem, at first glance, to violate the spirit, if not the letter, of those laws. Take living cells. In recent years, scientists have found that some molecular machines—proteins that perform crucial tasks of life, from shuttling molecules through membranes to reading information off of DNA—seem to move spontaneously. These machines are likely powered by the random motion of water molecules in their environment, the "thermal noise" that thermodynamics insists is not available for doing work.

While some researchers debate how such machines work without breaking physical laws, other scientists have begun to exploit similar phenomena to create artificial molecular motors—nanomachines that imitate nature by putting randomness to work. "The idea is, let's take advantage of thermal noise, rather than fight against it," says Dean Astumian, a theoretical chemist at the University of Maine in Orono.

Researchers have just begun to build artificial nanomachines that perform simple tasks, such as moving molecules, by steering random motion in one direction rather than another. In the Feb. 13 *Journal of the American Chemical Society*, a team led by David Leigh, a chemist at the University of Edinburgh in Scotland, describes the first molecule designed to use chemical energy to open or close a gate and allow one of its parts to randomly cross the gate in one direction, but not the other.

It's very much like the task assigned to a hypothetical "demon" by the 19th-century Scottish physicist James Clerk Maxwell. His thought experiment was an early attempt to show how the second law defines group behavior and thus applies only to large numbers of particles.

MAXWELL'S ANGEL The second law requires that in any given activity, some of the expended energy will end up as waste heat.

For example, even an efficient power plant can lose half or more of its fuel's energy to waste heat. This waste heat cannot be recovered without expending more energy—and producing more waste heat—in the attempt.

Ultimately, waste heat manifests as random molecular motion, like the incessant hailstorm of water molecules buffeting proteins in a cell's watery guts.

"It's sort of like you're riding a bicycle and there's a Richter-12 earthquake going on all the time," says George Oster, a molecular biology theorist at the University of California, Berkeley.

It's hard to see how the molecular movements (called Brownian motion) produced by such violence could accomplish anything useful. Every second, a typical molecular motor will exchange millions of times as much energy with the environment through these random collisions as it will in the performance of its actual task, Astumian explains. But beginning in the early 1990s, scientists began to suspect that certain protein motors can perform their tasks not despite Brownian motion, but thanks to it.

One example is RNA polymerase (RNAP), an enzyme responsible for reading genetic information from DNA. RNAP latches on to a DNA double strand at the beginning of a gene, cleaves the two strands apart, and clamps around one of them. It then moves along DNA's bases—the A's, C's, G's, and T's that constitute the genetic code's alphabet—and assembles a corresponding molecular chain of RNA. The RNA molecule then acts as a template for producing proteins.

RNAP, however, does not always move forward. Brownian motion can push it either way. "It's like a zipper—it slides back and forth," says Evgeny Nudler, a biochemist at New York University.

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>
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Roger Kornberg, a structural biologist at Stanford University, and his collaborators first decoded the structure of RNAP in 2001, earning him the 2006 Nobel Prize in Chemistry. In the same award-winning papers, the team suggested that RNAP may be able to select the Brownian fluctuations that propel it forward and discard those that would set it back. That sounds suspiciously like a free lunch, but in fact, the laws of physics do not prevent it.

RNAP's secret lies in the fact that the second law is statistical in nature. At the scales of molecules, random fluctuations can temporarily create small amounts of seemingly "free" energy. Cells can take energy out of Brownian motion by selecting the favorable fluctuations and rejecting the others—very much in the spirit of Maxwell's demon.

Maxwell asked whether the random differences among the energies of particles could somehow be harnessed. He imagined a box filled with a gas and divided into two parts by a wall that didn't conduct heat. The wall had a tiny door, and standing by it, "a being whose faculties are so sharpened that he can follow every molecule in its course," Maxwell wrote in *Theory of Heat* (1871). This "demon" could open or close the door whenever a gas molecule approached, in such a way as to let the faster molecules cross in one direction only, and the slower ones in the opposite direction. After a while, the faster molecules would make one side of the box hotter than the other. Heat would flow in the "wrong" direction.

For decades, physicists argued whether such a demonic being could actually violate the second law. Ultimately, modern thinking goes, the energy that the demon's brain spends on processing (and

erasing) information about the particles would offset any recovery of waste heat, and thereby preserve the second law's validity.

So, molecular motors such as RNAP could work like microscopic Maxwell demons, using energy to select favorable fluctuations of energy when opportunities arise. In fact, RNA polymerase is so far the best-established example of a biological Maxwell demon, says Steven Block, a biophysicist at Stanford University.

But that doesn't mean it gets a free lunch.

When they decoded RNAP's structure, Kornberg and his team discovered that RNAP includes a system of two moving parts, located next to the site within RNAP where new RNA bases bind to the DNA template. When this two-part system folds, it falls onto the binding site like a trigger onto a bullet casing. Perhaps, some researchers thought, such a trigger pushes the newly formed DNA-RNA double strand forward by one step.

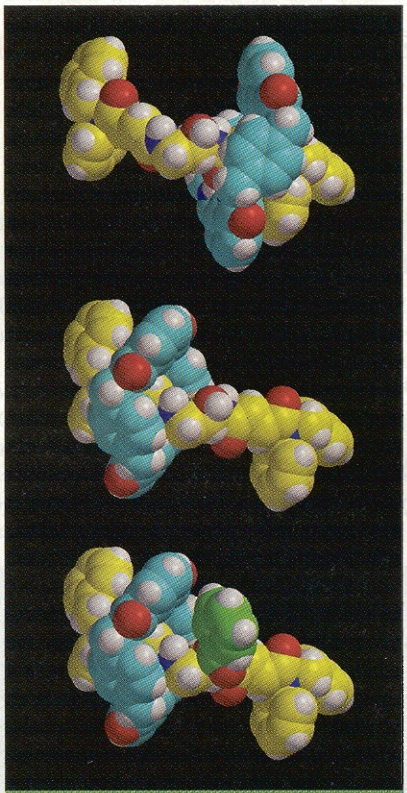
Indeed, in 2005, Nudler and his collaborators showed that mutations altering the trigger structure rendered the RNAP unable to move preferentially forward.

However, Kornberg suggests, the trigger may not be what pushes the zipper forward. Instead, the trigger's role could be to test the strength of the binding in the latest DNA-RNA base pair. If the wrong, noncomplementary RNA base had gotten there by mistake, it would not be bonded as strongly as a complementary base would be, and the trigger would dislodge it, correcting the transcription error. The trigger's "principal role would not be in motion, but in recognition," he says.

Here is where the Maxwell-demon analogy could be useful, Kornberg adds. Once a correct complementary base pair has formed, Brownian motion would allow the zipper to move forward. The trigger would prevent a backward step.

Block's team measured the pull exerted by single RNAP molecules during the transcription process. Those measurements seem consistent with this picture, Kornberg says.

So, Brownian motion would provide the energy for RNAP to crawl along DNA. The higher chemical affinity of complementary pairs—and the larger amounts of energy they release when they bind—would do the demon's work. And pay for lunch.



TAMING CHANCE — Thermal or Brownian motion moves a ring-shaped molecule (blue) from one side to another of a dumbbell-shaped molecule (yellow). But a "gate" molecule (green) is designed to lock the ring molecule to just one side of the dumbbell. Brownian motion provides energy to move the ring, but the gate molecule steers it.

S. GOLDUP/UNIV. OF EDINBURGH

GEOGRAPHY AS DESTINY No matter what the details of its machinery are, RNAP is an example of how evolution has invented ways of doing complex tasks in the forbidding environment of Brownian motion. Researchers who are trying to build artificial machines at the molecular scale—one of the promises of nanotechnology—would very much like to do the same, says Astumian.

The molecule described by Leigh's team at Edinburgh is a step in that direction, operating just like a Maxwell demon by opening and closing its gate to let molecules through.

"We made a molecule that works with the process that Maxwell envisaged," says Leigh, who proudly remarks that his house is just around the corner from the place where Maxwell once lived.

Leigh's molecule is really three molecules. Two form a type of rotaxane, which is a dumbbell-shaped molecule plus a ring molecule around the dumbbell's axle. Because of Brownian motion, this ring is generally free to bounce between the dumbbell's ends, where it can loosely bind. Left alone, the ring will keep randomly jumping between the two sides.

The researchers put their rotaxanes in water and added to the solution the third molecule, which is designed to bind to the middle of the axle. This third molecule would act as a gate, blocking the ring to one side and holding it there.

The ring's two sides have different shapes. When the ring is on one side of the dumbbell, the gate can bind to the axle. When the ring is on the other side, its shape will prevent the gate from binding.

The researchers demonstrated that in 70 percent of the molecules, the rings ended up sticking to the preferred side of the dumbbell, trapped into position by the gate.

The team described a similar molecule for the first time a year ago in *Nature*—although in that case, the gates were controlled by shining ultraviolet light on the solution rather than by the presence of molecules in the solution itself.

In both cases, the energy moving the ring comes from Brownian motion, but the molecules determine where the ring ends up. "It's a chemical way of implementing Maxwell's demon," says Astumian, who in 1998 envisaged a similar working principle with Imre Derényi, now at Eötvös University in Budapest.

Leigh says that one could imagine stringing together many rotaxanes. The rings would still move mostly at random, but on average the gates would tend to push them in a specific direction, from one rotaxane to the next.

EINSTEIN RULES Meanwhile, physicists, inspired in part by the discoveries about protein motors, have found renewed interest in the small fluctuations that characterize thermodynamics at microscopic scales.

"On average, the second law will never be violated," says Christopher Jarzynski, a theoretical physicist at the University of Maryland in College Park.

But, as Maxwell suggested, the second law may apply more to macroscopic thermodynamics. It thus is not always helpful for understanding phenomena such as the spontaneous folding of newly minted proteins, which take place in the cell's thermal bath.

In the 1990s, Jarzynski and others developed new theoretical tools to predict how much energy the Brownian bath can spontaneously make available, for example, to help out a molecular motor.

In 2002, Berkeley biochemist Carlos Bustamante and his collaborators tested Jarzynski's hypothesis for the first time on a biological molecule. They took single RNA molecules in a folded state and repeatedly pulled them apart to unfold them, while measuring the force exerted during the process. In accordance with Jarzynski's predictions, Brownian fluctuations would sometimes impede the process, and sometimes help it by providing a bit of free energy. In such cases, says Bustamante, "the work is being done by the bath, in a sense."

Last year, another team performed similar measurements by unfolding proteins (*SN: 7/14/07, p 22*). Experiments such as these can help researchers understand why biological molecules fold in one way rather than another—knowledge that may help them understand diseases caused by protein folding gone wrong.

In any case, it seems that the free lunches of molecular motors do always carry some sort of cost. Consequently, most scientists today would still agree with the sentiment Einstein expressed about thermodynamics in 1949: "It is the only physical theory of universal content which I am convinced that, within the framework of the applicability of its basic concepts, will never be overthrown." ■