How Molecular Motors Extract Order from Chaos
(A Key Issues Review)

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Abstract. Molecular motors are the workhorses of living cells. Seemingly by
"magic", these molecules are able to complete purposeful tasks while being immersed
in a sea of thermal chaos. Here, we review the current understanding of how these
machines work, present simple models based on thermal ratchets, discuss implications
for statistical physics, and provide an overview of ongoing research in this important
and fascinating field of study.

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1. Introduction: From thermal chaos to biological order

As physicists, we know that physical principles govern all material systems. When it comes to living systems, however, we often encounter difficulties explaining the complex, "purposeful" activity that can be found in living cells. The famous biophysicist Max Delbrück expressed the difference between biology and physics as follows: "...every biological phenomenon is essentially [a] historical one, one unique situation in the infinite total complex of life. Such a situation from the outset diminishes the hope of understanding any one living thing by itself and the hope of discovering universal laws, the pride and ambition of physicists. The curiosity remains, though, to grasp more clearly how the same matter, which in physics and in chemistry displays orderly and reproducible and relatively simple properties, arranges itself in the most astounding fashions as soon as it is drawn into the orbit of the living organism. The closer one looks at these performances of matter in living organisms the more impressive the show becomes. The meanest living cell becomes a magic puzzle box full of elaborate and changing molecules ..." [1].

Even today, physicists often do not know how physics relates to fundamental life processes. Many consider biology as a type of elaborate "stamp collecting" - to paraphrase a quote often attributed to Rutherford. At the same time, many biologists are not familiar with the necessary physics to base biological function on fundamental physical processes. Our modern understanding of biological physics therefore bridges a crucial gap in understanding.

We now know that Delbrück's "magic puzzle box" is full of molecular machines and motors - little machines that perform purposeful tasks while surrounded by violent thermal chaos. Molecular motors take on a number of tasks in our cells, including moving cargo, re-arranging the cell's cytoskeleton, transporting organelles, moving chromosomes during cell division and helping cells change shape [2, 3, 4, 5]. A close look into a living cell is indeed like looking into a world of magic, a feeling well captured in a video produced at Harvard University called "The inner life of a cell" [6]. It is tempting to invoke special life forces to explain the emergence of purpose in biology, but, clearly, we know that a physical explanation must be behind these startling observations [7].

To get to grips with the seemingly non-physical behavior of living systems, we need to boil down the problem to its most basic physical ingredients. The most basic entity of a living cell is a molecule and, consequently, the question we have to answer is: How can a single molecule create directed motion in the presence of strong thermal fluctuations? Or more precisely, how can a molecule, immersed in thermal noise, but provided with some kind of low entropy energy (either in the form of chemical or electrostatic energy) perform directed work? How does chemistry turn into "clockwork"?

These questions have begun to be answered in the last two decades. While the details differ for different molecular machines, general principles can be deduced. These general principles touch on fundamental physical questions such as the meaning of entropy, free energy and the second law of thermodynamics at the single molecule level.
They connect biology to statistical mechanics and even information theory, and present questions that are not encountered in other areas of physics. In this review, we will review the history of our understanding of these connections, understand the general theoretical framework to understand molecular machines, and see how real biomolecular machines measure up against this framework. We will also briefly review the physical techniques used in teasing apart the detailed mechanisms of molecular motors, as well as present a summary and outlook.

2. A very brief prehistory of molecular machines

To give a full review of the history of our physical understanding of molecular machines would be impossible, and I apologize if I leave out any important players. Nevertheless, a general outline can be given. We start with a definition of molecular machines: A molecular machine is a molecule or small molecular assembly that performs a function that locally increases free energy or performs work at the expense of chemical energy, while in the presence of significant thermal fluctuations. Typically the thermal fluctuations are of similar magnitude as the chemical energy used and the work produced. Molecular machines are capable of repeatedly transforming one form of energy into another, while using random thermal motion as part of their energy input. In this paper, we will primarily focus on a subtype of molecular machines, molecular motors, which are machines that convert chemical energy into directed mechanical work. However, many statements about molecular motors also apply to molecular machines at large and the boundaries are not always well defined. For example, a well-known molecular machine, ATP synthase, converts electrical energy into mechanical energy, which is then transformed into chemical energy [8]. While ATP synthase is not strictly a molecular motor, it incorporates mechanical motion as part of its working cycle, and increases free energy by "recharging" ADP to ATP.

The importance of strong thermal fluctuations makes molecular motors very different from macroscopic motors. They cannot be described by macroscopic thermodynamics, but have to be described statistically. The strong presence of thermal fluctuations makes the world they inhabit very different from our macroscopic world, where such fluctuations are often averaged out and invisible. For example, it has been pointed out that while a macroscopic object at rest will stay at rest in absence of an external force, molecular machines are subject to random forces and the "natural state" is random diffusion. At the nanoscale, a force must be applied to keep an object from moving [9].

The study of molecular motors began in physiology with the study of muscles. The most cited work of this era is the work by Archibald Hill from the late 1930s [10]. Hill performed extensive experiments on isolated muscle tissue to measure the work and heat output under various load conditions, from no load to isometric load (i.e. a load large enough to prevent the muscle from contracting). An important result was the "Hill curve", which relates applied load to the speed of contraction. The equation
describing the curve is given by \((P + a)(v + b) = (P_0 + a)b = \text{const.}\) Here \(P\) is the applied load, \(v\) the speed, \(a\) the frictional force per unit area against which the muscle performs work, and \(b\) represents a characteristic velocity constant. \(P_0\) is the stall force, which Hill found to be equal to 4 times the frictional force. He also determined that \(b\) (which is the characteristic rate constant in the equation) was temperature dependent with a 2.08 fold increase every 10°C, corresponding to an effective energy barrier of 22-23 \(k_B T\) at 300-310K. Another important result was the observation that the heat generated was independent of load, but only depended on the length of contraction. This observation suggested that a fixed amount of chemical energy was transduced for each "step" of muscle contraction. The numbers that Hill measured for a frog muscle provide reasonable estimates for forces and speeds at the molecular scale. Taking Hill’s numbers and scaling them down for a "muscle" of 10 nm^3, the maximum speed is about 130 nm/s and the stall force of order 14 pN. These are not unreasonable numbers compared to modern measurements on single myosin molecules, where a single myosin molecule may generate 3-4 pN and the translocation speed of an actin fiber by a single myosin is about 3000 nm/s [11] - considering that in real muscle, many myosins work together, thus increasing the force per myosin and decreasing the speed.

How muscles work at the microscopic scale was impossible to tell until the advent of sufficiently powerful microscopy methods, such as electron microscopy, in the early 1950s. Groundbreaking work was done (independently) by the two "Huxleys": Hugh and Andrew, and their collaborators[12, 13, 14]. Hugh Huxley, together with Jean Hanson, were among the first to apply electron microscopy and X-ray diffraction to the structure of muscle cells and formulated the "sliding filament hypothesis", which stated that there were two types of interdigitated filaments, which slid past each other during muscle contraction[15]. These were linked during rigor by "crossbridges". This was the basis for Huxley’s later formulated hypothesis of the "tilting" or "swinging" cross-bridge model, in which the cross-bridges first form (myosin attaches to actin), swing to propel the actin forward and detach again [16]. Andrew Huxley supplemented this work with physiological observation, and provided the experimental proof for the "sliding filament" hypothesis in 1966 [17].

Early work on muscles was inspired by experience with human-built machinery. Before the sliding filament hypothesis, muscle was believed to work by the coiling of springlike proteins[18]. The tilting crossbridge idea of Hugh Huxley was also quite mechanical and, while physiologically correct, did not clearly explain how energy was transduced from chemical to mechanical form. Andrew Huxley’s work was the first that hinted that something quite unusual may be going on, something that places molecular motors outside the familiar thermodynamics and mechanics of macroscopic machines: In Andrew Huxley’s paper of 1957[19], he advanced a scheme in which the chemical energy used up by muscle was not used to move the fibers relative to each other. Instead, in his words, "the sliding members can combine temporarily with sites on adjacent actin filaments, the connection being formed spontaneously but broken only by a reaction requiring energy to be supplied by metabolic sources." In other words, energy was used
to break the connection between myosin and actin, not to move the myosin and actin fibers past each other. How, then did the fibers move? Huxley envisioned that the myosin heads were attached to an elastic backbone, while being randomly moved by thermal motion when not attached to actin. This thermal motion would, statistically, allow the myosin to occasionally reach an attachment point on the actin, while stretching its elastic backbone - temporarily storing thermal energy as elastic energy. Once the myosin would bind to the actin, the stored elastic energy would allow the myosin to pull the actin. A change in conformation of the myosin as it relaxed would then allow the chemical energy "from [a] metabolic source" to be transduced and the myosin would detach from the actin to repeat the cycle. Such a cycle has been shown to work in principle[20].

It is interesting to consider what would happen if the molecule would not detach from the track after pulling it. Continuing to be subjected to thermal fluctuations, the myosin and the attached actin would fluctuate back-and-forth, not making any headway. Thus, somewhat paradoxically, letting go of the filament is a crucial step in producing directed motion.

It does not seem that the novelty of this suggestion was fully recognized at the time. What Huxley was suggesting was that muscle transduced random thermal motion into directed mechanical motion, and that chemical energy merely served to reset the molecular machine to its starting position. In other words, the machine was capable of spontaneously rectifying random thermal motion due to its asymmetric construction (the attachment point was located at an offset from the equilibrium position of the molecule). This seemed to violate the second law. However, the machine could not do this repeatedly, as it must first detach to repeat the cycle. On average, detachment requires energy. Thus, externally supplied energy must be supplied to "reset" the machine and allow it to repeat the cycle. This energy is ultimately converted into heat in accordance with the second law. However, the machine could not do this repeatedly, as it must first detach to repeat the cycle. On average, detachment requires energy. Thus, externally supplied energy must be supplied to "reset" the machine and allow it to repeat the cycle. This energy is ultimately converted into heat in accordance with the second law. This suggests a rather interesting statistical formulation of the second law of thermodynamics, which goes back to the early pioneers of statistical thermodynamics, but is usually not mentioned in the average thermodynamics course: The second law does not prohibit the existence of some mechanism that can directly convert heat into usable work at isothermal conditions or even using a lower temperature reservoir, but it does prohibit a mechanism that can do this continuously [21].

Another observation from Huxley’s model is that molecular motors work by switching between states with low energy barriers, which allow diffusion, and states with high energy barriers (such as strong binding to a filament), which "fix" the location of the motor from time-to-time. Breaking the high energy barrier states requires an external input of energy.

The new idea of a machine that can rectify thermal motion was seriously considered by biophysicists only 30 years later, when techniques to observe single molecular machines became available. Measurements using these new techniques showed that molecular machines moved statistically and even exhibited occasional backward steps, compatible with a thermal noise driven process [22]. Until then, this remained an
interesting, but far from mainstream hypothesis, and more conventional mechanical models dominated. It should be noted that many researchers believe today that muscle myosin (myosin II) operates quite close to Hugh Huxley’s ”powerstroke” model[23] and not as Andrew Huxley suggested (although there are dissenters [24]), but this does not mean that myosin is not well described as a machine that rectifies thermal noise.

The basic idea of a molecular machine rectifying thermal noise has proven extremely fruitful: It has led to a new understanding of the statistical nature of the second law, the creation of artificial machines and fluctuation-driven particle sorters and, as we will see, continues to be a valuable model for understanding molecular motors. For example, even through many researchers believe that myosin moves rather mechanically, there is no clear relationship between ATP hydrolysis and the powerstroke, but rather a not completely deciphered sequence of hydrolysis and various stages of phosphate and ADP release during the powerstroke[25, 23]. Also, due to the presence of varying activation barriers in the cycle, thermal activation plays an important role. Consequently, the boundaries between thermally driven and powerstroke driven machines are rather fluid[26, 27].

3. Fluctuations, Maxwell’s demons and ratchets

What Andrew Huxley suggested in 1957 and what subsequent studies of single molecules confirmed is the striking fact that molecular machines can rectify random thermal motion. Mechanical motion of these motors is caused by thermal motion as they traverse energy barriers, and the main challenge is to explain how they can convert random thermal motion into directed motion. This problem is reminiscent of an old paradox in thermodynamics - how the time asymmetry of the second law emerges at the molecular scale, knowing that molecular collisions are time-reversible.

At the end of the 19th century, the statistical nature of microscopic physics and the existence of atoms and molecules were still controversial. How the deterministic laws of thermodynamics could emerge from statistical laws was explored by a number of paradoxes and Gedankenexperiments - the best known being ”Maxwell’s demon” [28]: The demon is a microscopic creature which controls a trapdoor that separates two chambers filled with gas. Initially the two chambers are at the same temperature, but the speeds of the gas molecules are statistically distributed according to the Maxwell-Boltzmann distribution. The demon can open and close the trap door to sort slow and fast molecules into different chambers, thus moving heat from a colder to a warmer body without any expenditure of energy - in clear violation of the Clausius statement of the second law of thermodynamics.

Maxwell introduced this pesky denizen of the nanoworld to illustrate that the second law was not a law inherent in matter, but rather was a statistical law. At the time, this was still an open question, as many physicists did not accept statistical mechanics (based on random motions of atoms and molecules) as a valid base for the deterministic machinery of thermodynamics. One such skeptic was Gabriel Lippmann,
a French physicist, who was the first to suggest a mechanical equivalent to Maxwell’s demon that still serves as a model for molecular machines - the ratchet. However, he did not analyze the ratchet in any detail, but instead made the statement that the idea of constructing such a machine at the molecular scale would be absurd[29].

The idea of the ratchet was further developed by Smoluchowski, a proponent of the statistical basis of thermodynamics. Smoluchowski introduced one version of his ratchet in 1912 (a torsional ratchet)[21], but refined the idea in 1914 (using a linear ratchet), where he clearly showed why it would not work[30]. His ratchet worked as follows: A small particle, able to be suspended by Brownian motion, is attached to a linear ratchet. The ratchet allows the particle to move up but not down. This would be achieved by having a spring-loaded pawl pressing into asymmetric teeth on the serrated vertical shaft of the ratchet. The asymmetry of the teeth makes the force required to move up less than the force required to move back down. Thus, this machines could rectify thermal motion and do work against gravity. However, for it to work, the random thermal force on the small particle would have to be large enough to overcome the spring-force of the pawl of the ratchet. Thus the ratchet mechanism itself would have to be weak enough to be moved by thermal energy and be subject to the same thermal fluctuations. These fluctuations would allow the pawl to open from time to time, letting the particle slip back down. Statistically, the position of the particle would follow a Boltzmann distribution - with or without ratchet. The only difference would be an additional energy term originating from the potential energy of the ratchet’s spring.

Richard Feynman, in his famous lectures[31], converted the linear ratchet to a rotating ratchet attached to a paddle wheel through a shaft. Placing the ratchet and the paddle wheel into different compartments, he showed that the ratchet will not move directionally if the wheel and the ratchet are at the same temperature for the same reasons already discussed by Smoluchowski. However, if the temperatures are different, the ratchet mechanism is able to rectify the random thermal collisions acting on the paddle wheel. This is because we can arrange a situation where the thermal fluctuations of the ratchet are reduced (by keeping it cool), while the paddle wheel receives enough energy from collisions (by keeping it hot) to push the wheel forward against the force of the spring. Interestingly, in the opposite case, if the ratchet is hotter than the wheel, the machine runs backward.

In a real molecular motor, the needed temperature gradients cannot be sustained: Over the small distances involved, any temperature gradient would be equalized in a time much shorter than the cycle time of the motor. Thus molecular motors cannot be based on temperature gradients, but must operate isothermally. However, Smoluchowski and Feynman showed that a simple ratchet in a uniform temperature bath would not be able to rectify motion, as all the parts of the ratchet would be thermalized equally and perform random motions. Thus how could a molecular motor work?
4. How molecular motors work - in principle

Theoretical studies have converged on two essential ingredients to rectify Brownian motion: Asymmetry and out-of-equilibrium conditions. The first is obvious because in a completely symmetric situation, there would be no preference to move in either direction, and the motor would just randomly diffuse. The second condition is less obvious. Feynman’s ratchet only worked in the presence of a thermal gradient. This thermal gradient would have to be maintained “artificially” to keep the machine working, i.e. the system has to be kept out of equilibrium. For molecular motors, thermal gradients are not possible, thus we need a different way to keep the motors out of equilibrium.

In chemical systems at equilibrium (or “steady-state”), forward and reverse reaction rates are equal. This is the principle of “detailed balance”. Looking at a molecular motor as a chemical machine, this would suggest that, at equilibrium, forward and reverse motions would proceed at the same average rate. Thus, a chemically driven molecular motor would make no headway, even in the presence of a spatially asymmetric potential. This shows that the system has to be kept out of thermodynamic equilibrium. Non-equilibrium conditions provide temporal asymmetry, as the evolution of the system in one direction moves the system closer to equilibrium while the opposite evolution moves it further away.

We can look at the necessity of temporal asymmetry and non-equilibrium conditions in a different way as well: It has been shown that a microscopic system can spontaneously fluctuate in a direction away from equilibrium. We have already suggested that, occasionally, a machine, such as Huxley’s myosin model, can convert heat into work against the demands of the second law. How often this can happen is given by the Jarzynski equality[32], which states that the Boltzmann-weighted average work performed by a ”one-shot” machine is equal to the Boltzmann-weighted free energy difference, i.e. that we have $e^{-W/kT} = e^{-\Delta F/kT}$. According to the second law, the work performed during a process should be less than the available free energy difference. However, Jarzynski’s equality suggests that this is only true on average, and that there are occasional times when the work equals or even succeeds the available free energy difference. How can the work exceed the available free energy difference of the process? Only if, occasionally, the motor gets a ”push” in the right direction from thermal fluctuations. Jarzynski’s equality tells us how often this happens statistically.

For molecular motors, this means that, given sufficient spatial asymmetry, we could construct a motor that could rectify thermal motion, but only in a one-shot fashion [21]. For example, in Huxley’s model, the motor could attach and pull the actin filament, but then it would be ”stuck”. The cycle could only be repeated if we provided energy to dissociate the motor from the filament. Thus, for repeated rectification, some kind of reset step is needed. This reset step requires external energy input into the system.

This is a powerful confirmation that the second law is a purely statistical law, and that a ”one-shot” deviation is not a violation of the law. It would only be a violation if the machine could rectify thermal motion repeatedly without any external energy input.
Thus, as long as external energy is needed to reset the machine, the second law is obeyed. Spatial asymmetry can be achieved in various ways, such as by an asymmetric potential energy of the track on which the motor operates, or by internal conformational changes that can bias the motion in a specific direction. Typically, both play an important role in real machines. Temporal asymmetry through out-of-equilibrium conditions can also be achieved in different ways: For example, by applying an oscillating force, by subjecting the motor to an oscillating potential [33], or by immersing the molecule in correlated, non-white noise [9]. Interestingly, it is not always apparent in which direction the motor would move - it does not necessarily move in the direction of less force resisting its motion. For example, given the same asymmetric potential, a motor based on an oscillating potential will move in the direction of the steepest increase (larger force) in the potential, while a motor based on oscillating force will tend to move in the opposite direction [33].

Coming back to Huxley’s model for myosin, where do we find the temporal asymmetry needed to make it work? Clearly, the machine only works if ATP hydrolysis is correlated with the conformational state of the machine, i.e. hydrolysis should only occur when the machine is attached and has pulled the actin filament - and not at any other time during the cycle. There must be a mechanism to ”gate” the hydrolysis to only happen at a certain time. In a real machine, this would be achieved by conformational changes, i.e. allostery. Moreover, we would not want the reverse process to happen, i.e. back-conversion of ADP to ATP upon binding. This can be avoided by keeping a large, out-of-equilibrium supply of ATP and a small concentration of ADP. Thus asymmetric structures, gating and non-equilibrium concentrations of energy-supplying molecules are the ingredients that make molecular machines work.

Finally, it should be noted that molecular machines in seemingly spatially symmetric structures have been demonstrated, but these typically involve a traveling or positionally switching potential. These machines introduce asymmetries in a different way, by mixing spatial and temporal asymmetry [34].

4.1. Correlated noise

A ratchet can be implemented when a particle, placed in an asymmetric periodic potential, is subject to a correlated noise in addition to the uncorrelated thermal noise. This was shown by Magnasco in two important papers on the subject. In a first paper [9], Magnasco was able to show that in the presence of ”white” thermal noise alone, an asymmetric potential is not sufficient to impose directed motion. However, the presence of any correlated noise with time correlations longer than molecular relaxation times (which are of order 10-100 ps) destroys detailed balance, and directed motion emerges in the presence of an asymmetric potential. As Magnasco points out, this creates directed motion ”for free”, as no particular energy input is needed as long as there is a source of correlated noise. However, correlated noise suggests low entropy noise, and, in a real physical system, such a noise would have to be generated by degrading a low entropy
Consequently, in a second paper [35], Magnasco showed that correlated noise can be generated by a chemical cycle, such as the binding and hydrolysis of ATP, and release of ADP. However, the chemical cycle would only serve as a ”correlated noise generator” and would not drive the motion directly. According to Magnasco, a molecular motor works by ”gluing together” a chemical cycle and a mechanical cycle. The chemical cycle ”eats non-equilibrium chemical energy” and produces ”correlated noise”, and the mechanical cycle ”eats correlated noise” and produces directed motion.

In such a scheme, chemical energy consumption is neatly separated from the mechanical cycle. In other words, the mechanical cycle does not consume any energy. Instead, the energy is consumed by the noise-generating chemical cycle. This represents a ”continuum” version of our description of a machine operating through a ”reset” step. In the reset step picture, chemical, low entropy energy is used for the reset, but not for the motion itself, and, here, low entropy energy is used to create correlated noise, but, again, not to drive motion directly.

Does this conceptual picture match actual observations? Yes, it does. As mentioned above, Hill observed that the dissipated energy in muscle is independent of load. Thus while the mechanical energy per step increases (assuming the step size is the same under different loads, which it is), the dissipated energy in the muscle stays the same. Assuming that only the chemical cycle dissipates energy, this observation fits Magnasco’s conceptual picture quite well. Also, comparing different types of motors, the relative timing of various steps along the chemical and mechanical cycles can be quite different: For example, ATP hydrolysis may initiate detachment from the track in one motor and cause a conformational change when the molecule is already detached in another. This supports the idea that, conceptually, some kind of mechano-chemical coupling driven by chemical, correlated noise is sufficient to drive directed motion on an asymmetric energy landscape. This is not to say that different times of coupling or timing schemes may produce more or less efficient motors, only that whatever the timing and coupling is, directed motion can be expected.

4.2. Fluctuating potentials and fluctuating forces

Around the same time Magnasco developed his somewhat abstract, but powerful, model for molecular machines, more concrete ratchet-based models were also being explored. To distinguish models, a specific nomenclature developed. For example, the term ”Brownian ratchet” is often used for all molecular ratchets, but it is also used specifically for polymerization motors, where Brownian diffusion and biased monomer binding leads to the unidirectional extension of a polymeric filament, such as actin[36]. For molecular motors, the two most basic models are the fluctuating potential or ”flashing ratchets”, and the fluctuating force, or ”tilting ratchets”[33, 37, 38]. In flashing ratchets, the height of the periodic potential fluctuates. This model simulates a molecular motor which switches between weak and strong, or attached and detached, binding states.
Here it is assumed that in the weak binding or detached state, the motor can diffuse, before re-attaching tightly to the track.

The tilting ratchet is based on a fluctuating force. Since the potential is the integral of the force, this means that the entire periodic potential is tilted up and down during the fluctuations. This type of ratchet may better correspond to a powerstroke driven molecular motor. Both types of ratchets have found practical applications in microscale particle sorters [39].

4.3. Connections to information theory

The connection between molecular machines and Maxwell’s demon goes deeper than their common relative, the ratchet. An argument can be made that molecular machines have deep connections to information theory, as they show how the not-so-obvious import of ”information” in the form of reset steps (in the discrete picture of the machine) or correlated noise (in the continuum picture) can lead to transformation of thermal chaos into directed motion. The ratchet idea can therefore tell us something deep about the second law of thermodynamics.

Physicist have tried to exorcise Maxwell’s demon from the time it was invented. The most common solution is to use the fact that a thermodynamic cost is exacted when a bit of information is erased. A Maxwell demon would have to erase its immediate measuring memory to make a new measurement (even if it uses a permanent memory to keep track of all measurements, it would need to move the information from the measurement memory to the permanent memory, which involves erasure). As has been theoretically shown [40] and experimentally demonstrated [41], the average cost of erasing one bit of information is $k_B T \ln 2$. The same experimental study showed that, occasionally, less than $k_B T \ln 2$ of energy is needed to erase a bit and that the frequency with which this happens obeys Jarzynski’s inequality.

The idea of erasing information is most evident in the case of a flashing ratchet, where the potential on which the ratchet diffuses alternates between an asymmetric non-zero potential and a flat, vanishing potential. This corresponds to a situation where the motor detaches, diffuses freely and reattaches to a track periodically. In this scheme, the motor ”forgets” its position relative to the potential when it detaches. Then, when the motor re-attaches to the track, the particle ”takes a measurement” of the potential, which is erased again on the next cycle. Landauer’s limit would then predict that the average minimum energy required to do this would be a reset energy of $k_B T \ln 2$. Thus such a motor could not operate for ”free”.

A more extreme and intriguing case is to run a machine not by using the Landauer energy equivalent of information, but on destroying information directly, with no energy transformation. A recent paper suggests that this may be possible [42]. If it is confirmed, this would be the first time that an equivalence of information and thermodynamic entropy has been demonstrated - an equivalence, which is traditionally considered to exist by analogy only, rather than by physical reality. In that case, a machine could
be run on degrading any conserved quantity, not just energy. For example, a machine could be run on degrading correlation in a spin reservoir [42].

Magnasco’s correlated noise model can also be linked to information theory. In this case, we consider that correlated noise is information-rich, low entropy noise, whose creation requires energy input.

4.4. Langevin and Fokker-Planck equations

Mathematically, the motion of a real molecular motor in the overdamped regime (which is the appropriate regime in this case) can be described by the Langevin equation:

$$\gamma \frac{dx}{dt} = -\frac{\partial}{\partial x} U(x, t) + \xi(t)$$

(1)

where $\gamma$ is a friction coefficient, $U(x, t)$ is the time varying, periodic potential, and $\xi(t)$ is an uncorrelated Gaussian thermal noise term, with $\langle \xi(t)\xi(t + \tau) \rangle = 2\gamma k_B T \delta(\tau)$. Note that the units of the noise term work out to the unit of force, because $\int_{-\infty}^{\infty} \delta(t) dt = 1$, i.e. the units of the delta function are formally $s^{-1}$.

If $U(x, t)$ is stationary and has an average slope of zero, the Langevin equation predicts that there is no net motion. This is the detailed balance case. If $U$ is stationary, but has a non-zero average slope, the particle will experience passive drift - for example in the presence of gravity or electrostatic forces. These are trivial examples that do not pertain to the discussion here. The more interesting situation occurs when $U(x, t)$ has no net slope, but changes in time. If this change is random and uncorrelated at the time scale of molecular relaxations, again no net motion of the molecule occurs, because the noise in the potential would just add to the uncorrelated thermal noise. Also, if the potential is symmetric in space, no net motion is possible for reasons already mentioned. However, if the time average $\langle U(x, t) \rangle_t$ has no net slope (and therefore the molecule is not subject to a net drift), but the potential is asymmetric in space and its fluctuations are time-correlated at times longer than molecular collision times, a net motion of the molecule can be produced. This is the basic set-up for a ratchet-like molecular motor.

The Langevin equation can be directly simulated using proper approaches used for solving stochastic differential equations [43, 44]. It is best to use a reduced representation of the Langevin equation, in which parameters like the friction coefficient are absorbed into the units of the equation. In that case, we can write:

$$\frac{dx}{dt} = -\frac{\partial}{\partial x} U + \xi(t)$$

(2)

As an example, let us consider the motion of a kinesin molecule, reduced to its simplest components. Kinesin molecules move hand-over-hand [45, 46] with a well-established step size of 8 nm of the entire molecule [47] (individual heads move 16 nm at a time). Energy for the motion is supplied by the hydrolysis of ATP, providing about 22 k_BT. Unbinding a kinesin head from the microtubule requires about 12 k_BT of energy [48]. However, there are likely other energy barriers to consider. Indeed, the observation that at physiological ATP concentration, the ATP turnover frequency is about 100
s$^{-1}$, suggests an effective barrier of about $23 \, k_B T$. If we use $k_B T = 4.3 \, pN \, nm$ as a characteristic energy scale, and the step size of $8 \, nm$ as our length scale, a characteristic force is given by $k_B T/ (8 \, nm) = 0.54 \, pN$. To establish a characteristic time scale, we need to obtain an estimate for the effective friction coefficient. Following Astumian [33], we can use the stall force and average speed at zero load to estimate the friction coefficient. The argument is that the motor will stall when the load force equals the force the motor can generate against friction in the absence of a load, i.e. $F_{\text{stall}} \approx \gamma v_{\text{avg}}$. Recall that Hill showed the two to be related by a factor of 4, which we will neglect here in the interest of simplification. A typical speed for kinesin under zero load is 700 nm/s, and a typical stall force is 7 \, pN [49]. Using these values, we obtain $\gamma = 0.01 \, \frac{pN}{nm}$. Normalizing this to 1, we obtain a characteristic time scale of 0.15 \, s, i.e. $\gamma = 1 \left( \frac{0.54 \, pN}{8 \, nm} \cdot 0.15 \, s \right)$. As an aside, this friction coefficient is much higher than we would expect from Stokes friction in water and is almost entirely due to energy dissipated to the microtubule. A rather arbitrary parameter is the asymmetry of the potential, $\alpha$, for which we chose a value of 0.95, accounting for the strong conformational gating in these motors.

Having these units in place, we can set up a simple model for a moving kinesin molecule, following Astumian [33]. We assume that the potential is spatially periodic, piece-wise linear and continuous, but asymmetric with linear slopes forming the ”teeth” of a linear ratchet. The potential’s asymmetry is described by the asymmetry parameter $\alpha$, which is equal to 0.5 for a fully symmetric system. When stationary, the teeth have a height of $E_0$, which we equate with a reasonable energy barrier somewhere between the barrier to dissociate from a microtubule and the estimated barrier from the turnover frequency. We chose 19 \, k_B T. We will use the flashing ratchet model (oscillating potential), as it better describes what is actually happening: As a kinesin motor domain dissociates from the microtubule, the potential it ”sees” is diminished in height. The energy supplied by ATP is more than sufficient for dissociation. The fluctuations in potential are given by the energy provided by ATP hydrolysis, i.e. 22 \, k_B T. The time dependence of the fluctuations is given by a dichotomous symmetric Markov process. Using these parameters, we can simulate the motion of kinesin molecules. Some typical trajectories are shown in Figure 1. These trajectories are very similar to what may be found in an experiment. However, the predicted motor speed at the observed ATP turnover frequency of kinesin (about 100Hz), is smaller ($\sim 150 \, nm/s$) than actually observed (700 nm/s).

Figure 2 shows the speed of the flashing and tilting ratchets as a function of the average turnover frequency. The turnover frequency is changed in our model by adjusting the transition probabilities between the positive and negative states of the Markov chain fluctuations according to

$$
\langle T \rangle = \frac{1}{\langle f \rangle} = 2 \sum_{n=1}^{\infty} nP^n = \frac{2P}{1 - P^2}
$$

where $P$ is the probability that the system will stay in the same state (i.e. $P = 1 - P_{\text{switch}}$) and $\langle T \rangle$ is the average period and $\langle f \rangle$ the average frequency of the noise.
Figure 1. Sample trajectories for the flashing ratchet at different average flashing frequencies.

Figure 2. Dependence of the speed of tilting (a) and flashing (b) ratchets on ATP turnover frequency. For the tilting rachet, parameters matching myosin II were used, whereas for the flashing ratchet, parameters matching kinesin were used.

It can be seen from Figure 2b that an optimal speed for the flashing ratchet (using kinesin parameters) is obtained at a turnover frequency of about 5600 Hz, which is higher than the 100 Hz frequency of actual kinesin molecules. Also, at the known turnover frequency of a real kinesin molecule (about 100 Hz), the predicted speed is less than is actually observed. Thus, real molecular machines are more efficient than the diffusing ratchet model used here. This is likely due to the much better "gating", i.e. the avoidance of back-slipping, which is achieved by the combination of internally choreographed conformational changes and the fact that most types of kinesin have two motor domains working in a synchronized fashion. Using a tilting ratchet model for kinesin (not shown) did not help to obtain a better agreement, as the maximum speed at a certain frequency is limited by friction.
On the other hand, using a tilting ratchet for myosin (Figure 2a) provides a surprisingly good match to actual observations. To implement this model, we assumed a "tooth" height of 12 k_BT for the potential and a 22 k_BT tilting potential (from ATP), resulting in a tilting force of 9 pN with a step length of 11 nm. The characteristic force scale was k_BT/11nm = 0.39pN. The effective friction coefficient was determined to be γ = 1.1 × 10^{-3} pN nm, using a stall force of 3.4 pN [11], leading to a characteristic time constant of 0.013 s.

At a 300-400Hz turnover frequency, the predicted speed matches the speed of real myosin II molecules quite well (2000-3000 nm/s). Moreover, for the tilting ratchet model for myosin, the dependence on load force as shown in Figure 3 predicts a Hill curve, however with fitting parameters that are different from what Hill measured. While the frictional force, a, matches quite well, the stall force is about 1/2 of what Hill measured, and the characteristic speed of a single myosin is much higher than what Hill found in his bulk measurements. As mentioned, this is most likely due to the fact that in real muscles many motors work in parallel.

The fact that, for both ratchet models, the speed tends to zero at high frequencies is easily understood. High frequency noise approaches random white noise because correlation times approach molecular collision times. Also, at high frequencies, the motor does not have time to diffuse from a minimum of the potential to the next nearest maximum during a single cycle. This suggests that the critical time corresponds to the average time it takes for the motor to diffuse far enough. The average time needed for a motor to diffuse a distance x is equal to t_D = x^2 / 2D, where D = k_BT / γ is the diffusion coefficient. The distance from a minimum to the next nearest tooth depends on α. For α = 0.95, we have x = 0.05 in reduced units. Taking D = 1, we find t_D = 1.25 × 10^{-3}, which translates into a characteristic time of 0.19 ms for our kinesin model, i.e. a frequency of 5300 Hz. This is very close to the frequency at which maximum speed is observed in Figure 2 b.

At low frequencies, the tilting ratchet shows large displacement speeds, as the motor drifts while the potential is tilted. Since the potential is asymmetric, drift is stronger in one direction than in the other. For the flashing potential, low frequencies are associated with long periods of diffusion over a flat potential and therefore random diffusion dominates, reducing net speed. For flashing ratchets, optimal speed is achieved when the motor diffuses just far enough to reach the next peak of the potential when the potential is weak or zero, and then drifts down the incline of the potential, when the potential is strong again.

Although instructive and a good way to generate sample trajectories, directly solving the Langevin equation to obtain statistical averages can be tedious, and therefore the Langevin is often translated into the corresponding Fokker-Planck equation, which allows for the calculation of the probability distributions and averages without calculating multiple random trajectories. In the overdamped, one-dimensional case
introduced above, the Fokker-Plank equation takes the form:

\[ \gamma \frac{\partial P(x,t)}{\partial t} = \frac{\partial}{\partial x} \left( P(x,t) \frac{\partial U(x,t)}{\partial x} \right) + k_B T \frac{\partial^2}{\partial x^2} P(x,t) \]  

where \( P(x,t) \) is the probability of finding the molecule at position \( x \) at time \( t \).

Using this approach, the dependence of the speed on various parameters can be determined more easily, either by solving the equation for the steady-state case [33] or by using expansions [50]. In either case, the Fokker-Planck equation reduces to a set of linear equations.

How do these mathematical models relate to the information theory picture mentioned earlier? The connection is the fluctuating potential, which switches between a high barrier state ("measurement of the potential") and a low barrier state ("forgetting the measurement"), requiring external energy input to repeatedly switch from one to the other.

5. How molecular motors work - in reality

5.1. Energy conversion at the nanoscale

Molecular machines operate in a regime where thermal, chemical and mechanical energies are of comparable magnitude (within about 1-1.5 orders of magnitude in most cases). To build machines that have mechanical or chemical energies this close to thermal energies at physiological temperatures imposes a certain length scale on the machine. If the machine is too large, thermal energy will be too small to effect any mechanical deformations of the machine. For example, let us assume we would like to bend a beam...
of material. The energy required to bend a cube of material with edge length $a$ is given by $U_{\text{bend}} = \frac{Ea^3}{8} \epsilon$, where $\epsilon = \frac{\delta}{a}$ is the shear strain. Assuming 1% strain and $E = 1$ MPa (for an organic material), we find that for an object of 10 nm dimensions, the energy required is about 2 $k_B T$, while for larger objects, the bending energy is out of reach for thermal fluctuations. Similar calculations can be made for other types of energy, such as the energy required to charge an object (many proteins are charged in solution) or to break weak bonds. It is found that all these types of energy become similar to the available thermal energy only if the system size is in the 1-100 nm range. Thus, we can build autonomous, thermally driven machines only at the nanoscale [51].

5.2. Moving along a linear track

One distinguishing feature of biological molecular motors is that they essentially operate in one dimension, i.e. they move along a linear polymeric track. It can be speculated that they originated from proteins diffusing along polymers, such as DNA, RNA, actin or microtubules [52]. One-dimensional diffusion is common in biology, and, in the case of DNA and RNA, provides a way to perform fast searches for matching sequences, which would be near impossible with random 3D diffusion. To make a simple diffusing molecular motor, an asymmetric track is needed, as well as a source of correlated noise from the binding and dissociation of molecules. Most biological polymers are asymmetric, and enzymes, which bind and dissociate various substrates are common. It can therefore be easily seen how an enzyme bound to a polymeric molecule could turn into a simple directional motor and evolve from there.

5.3. Structure and conformation

The basic structure of a molecular motor consists of a motor domain or "head", which includes the catalytic domain where ATP is hydrolized (called the "ATPase"), and neck linkers that connect the head or heads to to stalk ("heavy chain"), which is a "coiled-coil" of alpha helices. The stalk connect the heads to a cargo, another head or forms a filament, as in the case of myosin II.

Absolutely essential for real molecular machines are chemically induced conformational changes, i.e. allostery. Allostery is the basic mechanism by which chemistry is translated into mechanics. Microscopically, allostery is associated with changes in the local conformation of a binding pocket, which, by a molecular lever mechanism, is amplified and acts on a distal part of the molecule. In enzymes, allostery is used to regulate activity, but in molecular motors, multiple allosteric changes help "gate" the motion to bias the motion forward and avoid backsliding, as well as time the different motions and binding events in the correct sequence. Allostery is also used in molecular motors to regulate their function, for example, by ensuring that they only attach to a filament and start walking when they are attached to a cargo [53].
5.4. Chemical & mechanical cycles and their kinetics

The typical cycle of a molecular motor has the following "ingredients":

1. ATP binding, hydrolysis, phosphate release, and ADP release
2. Binding and dissociation from a polymer track or filament
3. Internal conformational changes caused by and causing a) and b) (feedback)

The exact order in which (1) and (2) occur is controlled by conformational changes (3), but not always in the same order. Conformational changes provide the *mechano-chemical coupling*, i.e. the connection between the chemical and mechanical cycles of the motor. Because conformational changes are both caused by and result in different chemical and mechanical states, they provide feedback loops that gate the motion and ensure dynamic fidelity and consistency. Real molecular motors reveal significant complexity in terms of conformational states of the mechanical cycle and the kinetics of the linked chemical cycle. How these complexities can be conceptually reduced to gain a simplified ratchet model for a motor is not an easy task. But this does not mean that simple models as discussed here cannot provide insight into the basic physics of these motors.

5.5. Time scales

The relevant time scales and speeds of real molecular motors are determined by molecular friction, translational energy barriers and cooperative conformational changes. Therefore, while thermal molecular relaxation times are of the order of picoseconds, typical cycles in molecular motors are much longer - typically of the order of 1-10 ms. This is not surprising, as the real motion of a molecular motor is a highly choreographed, cooperative "dance" of a many weak bonds and molecular deformations.

5.6. Powerstrokes, thermal ratchets, loose and tight coupling

One of the most debated question is if molecular machines work by a thermal ratchet mechanism with a significant diffusional component, or more mechanically through a chemical energy driven "powerstroke". Taken as mutually exclusive ideas, both models have problems. Structural and mechanistic studies clearly show that many molecular motors exhibit *tight coupling* [54] between ATP hydrolysis and stepping (i.e. motors like kinesin [55] or myosin V [56] typically take one step per one ATP hydrolyzed). Also, stepping is associated with highly coordinated conformational changes that allow the motion to be highly choreographed and gated. All of this would suggest a pure "powerstroke" model.

However, there are some powerful arguments that this cannot be the entire story [57]. First of all, molecular motors do not directly convert chemical energy release into mechanical motion. The chemical cycling of ATP does not correspond to the mechanical cycling of the motor in the sense that there is no direct association between
ATP hydrolysis and the powerstroke. This would suggest an important role for thermal diffusion and support the reset picture described above. Indeed, it seems that ATP hydrolysis often causes conformational changes not directly associated with propelling the motor forward, such as "pre-strokes" or dissociation of a head from a track. Moreover, as mentioned, the relation between the chemical ATPase cycle and the mechanical cycle is different in different motors.

Yet, this does not in itself contradict the powerstroke model. Some of the energy released by hydrolysis may be stored elastically in the molecule [58]. Moreover, the ATP dissociation happens in several distinct steps: first ATP splits into ADP and phosphate (P), then the P is released, and finally the ADP is released. Thus the energy from the ATP hydrolysis may be used over several conformational steps of the molecule (although some of these steps require energy, rather than provide it, such as the release of ADP) and possibly stored over this time as elastic energy [59].

The most convincing argument that a pure powerstroke model that does not take into account thermal motion is inadequate are the strong thermal fluctuations themselves: Clearly, any conformational change of the motor involves a transition state and corresponding energy barrier. Also, large conformational changes effected by quite distal binding events are often involved. For the motor to overcome energy barriers and find the next accessible complex low energy state, thermal fluctuations are indispensable. The search for different conformational states during the cycling of the motor is a diffusional process in a multidimensional configurational space on a complex energy landscape. While not a one-dimensional diffusional process as in our simple ratchet model, these are diffusional processes nevertheless. Therefore, ratchet models provide a powerful conceptual framework that can be used in many more realistic models of molecular motors.

Simple ratchet models for molecular motors were more popular from 1990-2000 and their influence has somewhat waned since 2000 because of more detailed mechanistic studies that obscure the basic physics underlying motor dynamics. However, with the discovery of new types of motors, researchers have found that some machines are more tightly coupled than others, with some exhibiting significant amounts of diffusional randomness and loose coupling between ATP hydrolysis and mechanical stepping [57, 60, 61, 62]. Also, tight coupling does not negate a ratchet mechanism, as tight coupling can be achieved in a ratchet through conformational feedback or the concerted motion of two coupled heads as in kinesin or myosin V. Indeed, a number of sophisticated ratchet models have been shown to fit experimental results very well: This includes discrete stochastic models that take the various states and their kinetics specifically into account [63], combinations of continuous ratchet models with chemical kinetics [64], or models that add feedback control to a basic ratchet mechanism [65, 66]. There is little difference between a well-gated, highly anisotropic ratchet and a motor driven by powerstrokes [26].

Assuming that we had a pure powerstroke mechanism, we would still have to determine the role and influence of thermal noise. While we could assume that thermal
noise is mostly a nuisance, this would imply that the stability of each state is beyond the reach of thermal fluctuations. This in turn would mean that the relevant energy barriers were much larger than $k_B T$ and each transition would have to be powered by an external energy input. However, there are already three transitions that require significant energy input: Dissociation from the track, dissociation of ADP and the powerstroke itself. However, only one ATP is used per stroke in closely coupled, powerstroke driven motors. Moreover, most motors likely exhibit several additional sub-states. For example, kinesin may exhibit at least four sub-states\[67\] during its "powerstroke" alone. Block et al. give transition rates of the order of $165 \text{ - } 1900 \text{ s}^{-1}$ between these various states, suggesting that each transition requires at least $20 k_B T$ to overcome the transition barrier. At the same time, some transitions may release free energy, which could be partially stored, while some of it is dissipated. Thermal energy and conformational diffusion must be involved in helping the motor through these various transitions and find consecutive low energy conformations.

5.7. Processivity

Molecular motors can be roughly divided into processive and non-processive motors. Processive motors are motors that can stay attached to a filament for a long time while moving along it, resulting in large distances covered. An example is kinesin, which is a highly processive motor. Myosin II, on the other hand, is a non-processive motor. It attaches, tilts and detaches. In terms of their function, these differences make sense: Kinesins work alone and have to move cargo over large distances in a cell. Myosin II works in collaboration with large numbers of other myosin motors and is attached to a myosin bundle. Thus it has to grasp, move and release periodically.

In terms of structure, processivity requires that the motor stays connected to the track at all times. The ratio of the times it stays attached versus dissociated during a typical cycle is the motor’s "duty ratio". Kinesin is a high duty ratio motor, while myosin II is a low duty ratio motor. For kinesin, the high duty ratio is the result of the presence of two heads, whose motions are correlated and gated. While one head moves, the other is firmly planted on the track.

Just like in the case of tight coupling, processivity does not imply that the motor could not work via a diffusive ratchet mechanism. It just means that during the diffusion, there must be some method of keeping the motor from diffusing away from the filament. For example, in the one-headed kinesin KIF1A, loops are used to hold on to the microtubule during the motion \[68\].

5.8. Real motors in our cells

There are many different types of molecular motors. The best known are kinesin, myosin and dynein. Each of these has many different subtypes, which vary in function, structure, processivity, and speed. As an example, in 2000, 139 members of the myosin "superfamily" were known \[69\]. At the same time, 144 kinesin types across 31 species
were known [70]. Not surprisingly, there is a vast literature on these various motors. To mention a few examples: While muscle myosin II is a non-processive motor working in collaboration with other motors to contract muscles, other myosins, such as myosin V [71], are processive cellular transporters much like kinesin, except that they move on actin. The archetypical processive kinesin we have mentioned so far in this paper is kinesin-1. An example of a non-processive kinesin is the kinesin-3 species NcKin3, which has a duty ratio of only 0.03 [61].

Beyond molecular motors, there is a vast array of molecular machines, many of which use a chemical or electrical energy source together with thermal motion to effect motion. We already mentioned ATP synthase, where motion is driven by an electrical potential difference across the mitochondrial membrane. ATP synthase is related to the bacterial flagellar motor, a fascinating rotary machine in its own right [72]. Cells are full of molecular machines: Active pumps that pump molecules against their chemical potential gradients [73]; machinery for DNA replication and transcription [74, 75]; ribosomes for RNA translation [76]; the machinery to divide the cellular chromosome [77]; and machines for membrane fusion [78].

6. How we know: Molecular-scale techniques

Figuring out how molecular motors work in detail requires a multi-pronged approach of biochemistry, structure determination and a variety of motility techniques to measure the motion of motors under various conditions. These approaches guide each other and are often combined. For example, structural studies combined with motility assays inform the construction of mutated versions of motors, which have missing structures or structures that are modified in some way. Then the motility of these modified motors are studied and compared to ”wild-type” (unmodified) motors to determine the function of each substructure of the motor. Another way to combine techniques is the combination of biochemical kinetics and motility studies to study the mechano-chemical coupling in detail. Even with numerous studies now existing, we still don’t understand the detailed operation of many of these motors. This is a testament to their complexity.

In the following, I will give a very brief review of some of the major techniques used to tease apart the detailed workings of molecular motors. This overview is somewhat artificially divided into six different techniques, but as mentioned, these are often combined. Also, it should be noted that in terms of the provided references, I make no claim of completeness or of having necessarily chosen the most relevant or important studies. References should be seen as a starting point for further studies only.

6.1. Biochemical kinetics

Chronologically, the earliest studies of molecular motors involved bulk mechanical studies on muscle [10] and biochemical kinetics. Biochemical kinetics studies of molecular motors use the same techniques as used in studies of enzyme kinetics. This is
not surprising, as molecular motors are essentially mechanically coupled enzymes. Such
studies typically involve the measurement of product evolution following a suddenly
increased substrate concentration by using stop-flow measurements, or in more modern
methods, surface-active approaches such as surface plasmon resonance or fluorescence.
The substrate of a molecular motor is usually ATP, and therefore biochemical studies
focus on the action of the ATPase part of the molecule; and how ATPase activity is
modulated by binding to or dissociation from the molecular track, or application of force
to the motor.

One of the most influential biochemical kinetics papers is the paper by Lymn
and Taylor of 1971 [79, 80], which established the still widely accepted kinetic scheme
governing myosin II. The basic kinetic scheme established in the Lymn and Taylor model
showed that ATP hydrolysis happened in the weakly bound or dissociated state, and
that the rate-determining step was the dissociation of the products, ADP and P. Kinetic
studies have been performed for most molecular motors, such as kinesin [81, 82, 83],
and have confirmed that molecular motors are basically mechanically coupled enzymes,
which obey Michaelis-Menten kinetics [59]. Slow product release seems to be a universal
feature.

6.2. Structure

Structure determination is performed by a number of approaches, including sequence
analysis[84], electron microscopy[85, 86], and X-ray diffraction[85, 87] on crystallized
samples. In principle, atomic force microscopy may also be a suitable technique as it
can achieve very high resolution images of proteins [88]. The most common techniques
are electron microscopy and X-ray diffraction. These techniques are very sophisticated
and can provide an atom-by-atom structure of the molecule. By binding derivatives
of ATP or ADP that are bound irreversibly to the motor, the detailed structure of the
motor in various stages of its cycle can be determined in a sequence of ”frozen” moments
[89].

6.3. Motility assays

Directly observing the motion of single molecular motors proved initially to be very
challenging, as they are smaller than the resolution limit of optical microscopy. Nor could
the motion be studied by electron microscopy or X-ray diffraction, as these methods
only work on ”dead” molecules, i.e. molecules fixed in a crystal or mounted on a grid
under vacuum. To see the motion induced by molecular motors, researchers found
an indirect way: They attached fluorophores, which they then could track in a light
microscope. Owing to the limited sensitivity and resolution of fluorescence microscopes
before 1990, the first studies of molecular motor motion did not observe the motors
themselves at single-molecule level, but rather the motion induced by many myosin
motors on fluorescently labeled actin [90]. Actin filaments can be decorated with many
fluorophores and therefore provide a large signal.
The basic setup of these "motility assays" is to attach a sufficient density of molecular motors (for example, myosin) to a solid substrate in such a way that the heads of the motors point up. Then fluorescently labeled actin filaments are floated on top of the myosin decorated substrate. By controlling the ATP concentration, the speed and direction of actin filaments can be measured, and the dependence of the speed on ATP concentration measured. Similar motility assays have also been used for kinesin and microtubules[91].

6.4. Laser traps

Optical or laser traps work by using light pressure to suspend a small dielectric bead in the focus of a strong laser [92]. The simplest use of laser traps for studies of molecular motors is to trap a bead decorated with molecular motors in the focus of the laser and then let the motors move on deposited filaments [93]. Since the beads are relatively large (200 nm or larger), they can be followed in an optical microscope. This is essentially the reverse of the motility assays mentioned above. An improvement is to use interferometry, such that the bead's position can be tracked with higher accuracy [47].

In a cell, motors operate under load as they move cargoes or re-arrange the cytoskeleton. To measure the effect of these forces, researchers exploit the fact that the laser focus provides a parabolic potential to the bead and therefore a constant stiffness "spring" with a typical spring constant of order 0.01 pN/nm. By moving the laser beam, small forces of 0.01 to several pN can be applied. This is used to measure the relationship between applied load and the motor's speed [22], as well as the ATP turnover rate [49]. Another use of laser traps is the measurement of the stall force [11, 22], i.e. the minimum applied force that will keep the motor from moving.

Double laser traps have also been used - for example, to hold a filament over a firmly attached motor-decorated bead in a reverse arrangement. This was used by Finer at al. to measure single steps and the stall force of the myosin II - actin system [11].

6.5. Super-resolution techniques

While laser traps have been very successful in elucidating the speed and force dependence of motors, they are not well suited to distinguish intermolecular motions. Initially as an alternative to laser traps, but then used for studies of the detailed stepping sequence of molecular motors, super-resolution fluorescence techniques have been increasingly used. Super-resolution fluorescence microscopy relies on the fact that, while the point-spread function of the diffraction spot of a single fluorophore has dimensions of about half the wavelength of the used light, the center of the diffraction spot can be located within a few nanometers using fitting techniques. Thus, super-resolution techniques do not directly provide images that are better than the diffraction limit, but they do allow tracking a fluorescent molecule with nanometer resolution. A requirement for super-resolution imaging is to image in the total internal reflection fluorescence (TIRF) mode. In TIRF mode, the illumination impinges on the backside of the glass slide holding the sample
at an angle beyond the Brewster angle, and thus the sample is illuminated only by an evanescent wave. This makes the detection highly surface sensitive (within 100 nm from the surface of the glass slide), reduces spurious signals and increases signal-to-noise for single molecule studies [94].

In studies of molecular motors, researchers attach fluorophores directly to the motor (rather than an attached bead). Fluorophores can be attached to different parts of the motor separately, such as the stalk and the motor domains. Finally, ATP can be fluorescently labeled. By using fluorophores that fluoresce at different wavelengths, the inter-play of ATP binding & dissociation, stepping and molecular movement can be observed in real time, and thus the chemical and mechanical cycles can be deciphered in detail [56].

6.6. Atomic Force Microscopy

Atomic force microscopy (AFM), invented in 1986 [95], is a now a quite common technique for high resolution imaging and force measurements. While more user friendly and versatile than laser tweezers as a force measurement technique, conventional AFM is not sensitive enough to measure the minute forces generated by molecular motors. The reason is that cantilevers are much too stiff (typical stiffnesses for cantilevers used on biological samples range between 30-100 pN/nm) and, with deflection sensitivities of 0.1 nm, minimum forces that can be measured above noise are typically in the 5-10 pN range. However, the recent development of ultrasensitive cantilevers has made it possible to push the force sensitivity much lower and kinesin stepping has been directly measured by AFM-based force sensing[96].

AFM can also be used to measure the mechanical properties of components of molecular machines by stretching. This technique has been applied, for example, to measuring the elasticity of the coiled-coil of myosin [97].

A truly spectacular and unique application of AFM to molecular machines was the direct high-speed, high resolution imaging of a single myosin V molecule during its motion along an actin filament [98]. Using a specially designed high-speed AFM with frame rates as low as 25 ms per frame (compared to typical frame rates of 1 minute/frame), Kodera et al. were able to create a movie of a myosin V molecule stepping along a microtubule, clearly resolving the movement of its two legs.

7. Current gaps and future work

Despite great progress in our understanding of molecular motors and machines, there is still much to be learned. While we have seen that we can deduce general principles of how molecular machines work, biology is a science of diversity and complexity. For molecular machines this means that every type of molecular machine works differently: Work cycles, regulation and structure can all be different. For example, it seems that while an external energy input (typically, but not always, in form of ATP) is needed to reset the
machine, the exact reset step can be very different from machine to machine. To deduce the exact work cycle of a molecular machine takes a number of experimental approaches and painstaking work. It is therefore not surprising that only a minority of molecular machines are reasonably well understood. Even studies of well-known machines like myosin [99] or ATP synthase [100] have not yet yielded a precise understanding of how they work. The exact details of the work cycle of a machine also influence its efficiency [101]. The question if molecular machines have evolved to maximum efficiency, and if not, why not, is also not resolved.

Beyond the mechanics and chemistry of individual machines, there are also many open questions about how machines are regulated [53, 102] and how they work collectively [38, 103]. There is also as yet only rudimentary understanding of the role of various machines in disease and aging [104, 105]. For example, a better detailed understanding of the gene repair and cell division machinery may help to explain why oncogenic mutations occur or why the fidelity of gene replication seems to degrade with age [105].

Another area of active investigation is the construction of artificial molecular machines, which have not yet reached the sophistication of their natural cousins[106]. Also, there are as yet few applications of such molecular machines, although one can envision various applications in nanoengineering[107, 108].

8. Summary and outlook

The study of molecular machines is of great importance in biology, but also has significant impact on physics. Models invented to explain how motors operate in the presence of large thermal fluctuations have provided new insight into statistical physics, the emergence of the second law of thermodynamics and connections to information theory. They connect to long-standing issues in statistical physics, going back to the founding of the field. The physics of molecular machines and motors continues to be fascinating and surprising. For one, it shows that noise is not ”bad”, but can be an essential ingredient of functional nanoscale systems.

The study of molecular machines has also motivated physicists to improve instrumentation and come up with new methods in areas such as laser tweezers, atomic force microscopy and super-resolution fluorescence microscopy. It has also stimulated research into creating artificial, thermally driven machines, which so far have found applications in particle sorting and will surely find many other applications.

The literature on molecular motors is vast. A quick search on Google Scholar with the keyword ”molecular motor” results in 2.16 million entries, with the top three papers cited more than a 1000 times each. There are many ways to approach these fascinating systems: What I tried to present here was how the study of molecular motors relates statistical mechanics to a fundamental understanding of how life works - in the process gaining a simple, but powerful framework for further study.

The study of molecular machines will continue to bear fruit: not only will we gain
a detailed understanding of the variety of molecular machines, but we will be able to
design some of our own - revolutionizing nanotechnology. Nature has been building
"nanobots" for billions of years - and now we have the opportunity to learn how she
did it. Theoretical studies of natural and artificial machines will continue to enrich our
understanding of statistical physics and guide further research.

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