Molecular motors: how to make models that can be used to convey the concept of molecular ratchets and thermal capture

Lindsay DoHarris, Amanda Giesler, Brent Humber, Aravin Sukumar, and Luke J. Janssen
Firestone Institute for Respiratory Health, St. Joseph’s Hospital, and Department of Medicine, McMaster University, Hamilton, Ontario, Canada

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DoHarris L., Giesler A, Humber B, Sukumar A, Janssen L.J.
Molecular motors: how to make models that can be used to convey the concept of molecular ratchets and thermal capture. Adv Physiol Educ 35: 213–218, 2011; doi:10.1152/advan.00107.2010.—A wide variety of cellular processes use molecular motors, including processive motors that move along some form of track (e.g., myosin with actin, kinesin or dynein with tubulin) and polymerases that move along a template (e.g., DNA and RNA polymerases, ribosomes). In trying to understand how these molecular motors actually move, many apply their understanding of how man-made motors work: the latter use some form of energy to exert a force or torque on its load. However, quite a different mechanism has been proposed to possibly account for the movement of molecular motors. Rather than hydrolyzing ATP to push or pull their load, they might use their own thermal vibrational energy as well as that of their load and their environment to move the load, capturing those movements that occur along a desired vector or axis and resisting others; ATP hydrolysis is required to make backward movements impossible. This intriguing thermal capture or Brownian ratchet model is relatively more difficult to convey to students. In this report, we describe several teaching aids that are very easily constructed using widely available household materials to convey the concept of a molecular ratchet.

BIOLOGICAL LIFE is a constant struggle against entropy: nearly every cellular process involves the expense of energy to work against the dissipation of concentration gradients, dispersion of molecules and even organelles, the disassembly of polymers into their constituent monomers, etc. In many of these processes, cells use a group of molecules collectively referred to as molecular motors, complex machines that convert various forms of chemical energy (hydrolysis of energetic molecules such as ATP, concentration gradients of ions, etc.) into kinetic energy and motion. Among the many examples of molecular motors, myosin may be the most familiar to physiology students, given the central role of certain myosin isoforms in mediating muscle contraction; other myosin isoforms are also important in the translocation of organelles and molecular packages from one part of a cell to another (10). In all these cases, myosin is viewed as hydrolyzing ATP and using that energy to walk along actin fibers in discrete steps. Kinesin and dynein are two other classes of molecular motors that also interact with another polymeric track protein (tubulin) to translocate organelles and various intracellular packages (e.g., chromosomes) throughout the cellular compartment (5, 6). Similarly, many polymerases actively translocate along a track: e.g., RNA and DNA polymerases and ribosomes move along strands of nucleic acids or proteins, inserting monomers and building up those polymers using the energy stored within ATP or some other high-energy molecule (8).

These various processes are generally portrayed as a concerted conversion of chemical energy to kinetic energy, torque and force, in much the same way that man-made motors use chemical or electrical energy to exert a dynamic force or torque on some lever or armature. This view is relatively easy to understand, relate to, and teach. However, another interpretation, one that is considerably more difficult to convey to students as well as to experienced colleagues, is that molecular motors might act as rectifiers that harness the constant and random vibrations within themselves and their substrates, allowing only those vibrations that are oriented in a certain desired direction and either suppressing other vibrations or redirecting them into the desired vector (1–3, 7, 9). For example, in the case of myosin, the actin and myosin fibers are constantly moving randomly due to their internal molecular thermal energy and the forces imposed by the connective tissue connections or the molecular payload on myosin. This Brownian motion causes the myosin head to constantly be swivelling back and forth, but ATP hydrolysis at the right moment in the kinetic cycle makes the backward motion thermodynamically unfavorable, thereby allowing only forward motions. As such, myosin effectively acts like a ratchet or filter, selecting out certain thermodynamic states and resisting others, and, in the process, rectifies random motion into a smooth forward motion, thereby using entropy to do work. Two fundamental differences between these viewpoints can be highlighted. First, the force that actually moves myosin along the actin fiber derives from ATP hydrolysis in the traditional model but from the thermal energy of the actin/myosin molecules themselves in the other model; hence, it is also referred to as the thermal capture or Brownian ratchet model. Second, the traditional model requires a great deal of torque to be exerted within the myosin neck region and transmitted through the globular head to physically translocate actin and thus pull on the cell membrane or move the payload attached to the myosin motor, whereas the thermal capture model requires the myosin head to merely form a stiff backstop to prevent the backward motion of actin.

This alternative view of the interaction between actin and myosin can also be applied to the movement of dynein and kinesin along their track protein, tubulin (5, 6). Moreover, polymerases in general can be reconsidered as molecular ratchets, converting random thermal energy into a concerted forward or synthetic motion (4, 8). That is, all three components of the synthetic complex [the polymerase, its molecular track...
(e.g., DNA in the case of RNA polymerase or mRNA in the case of a ribosome), and the growing polymeric strand are undergoing constant independent thermodynamic agitation, but the tight association between the polymerase and the track allows movements of the polymerase along the axis defined by the track itself but constrains movements in the other two orthogonal dimensions, and the growing polymeric strand further constrains that movement by acting as the backstop to prevent reverse motion along that axis. In this way, the polymerase moves progressively forward using random thermal energy, inserting monomers into the gaps created at the leading edge of the polymer and using the thermal energy liberated by the hydrolysis of ATP to create a stiff backstop.

As stated above, this concept of molecular motors as ratchets, capturing the random kinetic energy of the motor and/or of its payload to do useful work rather than burning up a separate fuel source to provide energy that is then exerted on the load, is a difficult one to convey to students. Below, we describe two alternatives, and there is great leeway in the dimensions and design of the teaching aids.

The two teaching aids described here are easily built from widely available household building materials and can be quickly assembled with a minimum of skill. Although we will describe the specific dimensions and particular materials to best guarantee success in the construction of these models, we must emphasize that once the instructor is well familiarized with the models and the principles that they can convey the components are easily substituted with amusing alternatives, and there is great leeway in the dimensions and design of the teaching aids.

The first and central component for both teaching aids is a source of high-frequency vibrations, which reproduces the intrinsic thermal energy of all molecules. We first used a battery-powered shaver (Fig. 1). However, alternatives include cell phones set to vibrate mode (Fig. 1); battery-powered electric motors with a small weight attached to the side of the armature to unbalance the motor as it spins, causing it to oscillate or quiver; children’s toys that wobble, etc.; in short, any object that can be induced to vibrate can be adapted for this teaching aid. Alternatively, we also used an electric power jigsaw with the blade removed or a variable-speed power drill with a weight affixed off axis to the drill bit to deliver vibrational energy directly to the tabletop on which the teaching aid was being used. A variation on this would be to have the students drum their fists around the edges of the table, although it will be important to ensure that they do so rapidly, asynchronously, and with similar strengths to best simulate random thermal vibrations.

The second component for this teaching aid is any stiff extension, which will rectify the random movements into a uniform vectorial translation of the vibrating unit: in other words, the ratchet (below).

Teaching Aids

Teaching aid 1. Our first design used an electric shaver and two paper clips (Fig. 1A). One paperclip was reformed into a pair of legs, which were taped to the body of the shaving unit close to the actual source of the vibrations (see Fig. 1B). The other paperclip was elongated and taped to the other end of the shaver to serve as a forward runner (Fig. 1B); the very leading edge of this runner was curved gently upward to provide as little friction as possible with the tabletop. Absent the paper clip legs, the shaver, when turned on, merely vibrated in place or skittered aimlessly. However, when the legs were attached, the shaver moved forward; minor adjustments to the angles of the legs were sometimes necessary to optimize that movement. After demonstrating this simple design to the students, we had them fashion their own models. One design used a cell phone, which vibrated when called (Fig. 1C) and gave qualitatively similar results.

Yet another design (Fig. 1D, left) proved to be particularly simple and yet useful in demonstrating the concept of a Brownian ratchet, the relationship between thermal energy and processive motion, and how

![Fig. 1. Assembly of the first design for teaching aid 1. A: the materials include an electric shaver and two paper clips, one bent to form a pair of legs and the other straightened out to form a forward runner. B: the legs were affixed with adhesive tape to the vibrating end of the shaver, while the runner was taped to the other end. C: the finished model. D: right: an alternative design in which a cell phone set to vibrate mode was substituted for the shaver. Left, two lengths of copper wire were used to construct another model (see Fig. 2 for more details), which used vibrations delivered to the tabletop to move.](http://advan.physiology.org/content/35/3/214.full.html)
small mutational changes can affect the function of the motor. Two 11-in. lengths of standard 12- or 14-gauge household copper wire (Fig. 2A) were twisted together for approximately half their length to form the front end of the motor (B). C and D, the remaining straight lengths were gently curved downward to form the rear legs; the front end was also gently curved upward to minimize frictional contact with the tabletop. Adhesive tape was used to affix an eraser on top of the legs (E and F). Vibrations were delivered to the tabletop using a power drill (see text for details). The direction of travel was determined by whether the feet contacted the table in front of (E) or behind (F) the center of gravity provided by the eraser (see text for details).

Using the model shown in Fig. 2, we had the students conduct some experiments to consolidate the concepts presented here. Students tracked the motion of the model by making markings on the table quivered in place during delivery of the vibrational energy. Its movement might be described by the general form of the Einstein-Stoke’s equation for diffusional mobility of a particle ($\mu$), as follows:

\[ \mu = \frac{D}{k_B \times T} \]

where $D$ is its diffusion constant, $k_B$ is Boltzmann’s constant, and $T$ is absolute temperature, but that is beyond the scope of this exercise. However, when the feet were pointed sharply downward and the legs bent back so that the feet contacted the table slightly behind the center of gravity provided by the eraser (Fig. 2E, note the dashed line), then vibrations in the rearward direction were hindered as the feet caught into the table, whereas vibrations in the forward direction were allowed. On the other hand, when the legs and feet were bent slightly forward such that the feet contacted the table just ahead of the center of gravity provided by the eraser (Fig. 2F), the apparatus now moved backward; this small structural change can be used to show how a relatively minor mutation in one part of a molecular motor can give rise to an entirely different class of processive motor, one that runs in reverse (e.g., such as kinesin vs. dynein). If the model travelled sidewise or in a circular path, a slight adjustment of only one leg was necessary to rectify the direction of travel.

Fig. 2. Assembly of the modified design for teaching aid 1. Two lengths of copper wire (A) were twisted together for approximately half their length to form the front end of the motor (B). C and D, the remaining straight lengths were gently curved downward to form the rear legs; the front end was also gently curved upward to minimize frictional contact with the tabletop. Adhesive tape was used to affix an eraser on top of the legs (E and F). Vibrations were delivered to the tabletop using a power drill (see text for details). The direction of travel was determined by whether the feet contacted the table in front of (E) or behind (F) the center of gravity provided by the eraser (see text for details).

Fig. 3. Quantifying the trajectory of the model. A: the model (i) was set in motion, and markings were made behind the leg at 2-s intervals (ii). At the end of the run, a line was drawn between the first and last markings to represent the mean trajectory of the model (iii). The portion of the experiment within the dashed box is expanded in B. We measured the distance between each marking parallel to the mean trajectory of travel (iv) as well as the distance of each marking perpendicular to the mean trajectory (v). These measurements were used to recreate the trajectory on an $x$-$y$ coordinate plot (see Fig. 4A) and to calculate the mean velocity of travel (see Fig. 4B).
Fig. 4. Data collected using the model in Fig. 2. A: recreations of the trajectories of the model at different drill speed settings (from lowest to highest speed, as indicated) with the model’s legs set into the forward mode (Fig. 2E; open symbols) or reverse mode (Fig. 2F; solid symbols). B: mean point-to-point velocity for the data shown in A with the model configured for the forward mode (open circles) or reverse mode (solid circles).

Fig. 5. Assembly of teaching aid 2. A: the basic components include a cardboard tube, four hairpins made from standard household electrical wire, several marbles, and a copper enclosure, which traps the marbles in a straight line. B: two copper hairpins are attached to the end of the cardboard tube in such a way as to direct the marbles out the bottom at an $-90^\circ$ angle. C: the other two copper hairpins are used as legs to lift the tube and $90^\circ$ elbow off the table. D: the entire model is made to straddle the copper enclosure and then loaded with marbles. When vibrational energy is supplied (in this case, by pressing a jigsaw to the tabletop), the “polymerase” propels itself along the track.
First, mount the tube onto the track and demonstrate that no forward movement occurs when the tube is not loaded with marbles, irrespective of how much vibration is imposed on the teaching aid. Next, load the tube with marbles. The marbles will stream out the bottom, roll along the track, and dissipate across the table, but there will be little, if any, net forward movement of the molecular motor irrespective of whether vibrational energy is supplied. This emphasizes the point that this molecular motor will not provide forward movement if there is not some form of a mechanism that resists movement in certain directions but freely allows movement in other directions (i.e., a ratchet). If, however, an impediment is put in place at the end of the track to prevent the marbles from rolling away, the model will now move forward along the track as the marbles drop into place with each forward vibrational movement of the unit and serve as backstops, which prevent any backward vibrational movement. This models the polymerase proceeding forward by capturing random thermal energy, inserting monomers into the gaps created at the leading edge of the polymer, and using ATP to lock the monomer into place, thereby forming a stiff backstop. Forward movement of the teaching aid will continue as long as the tube is kept supplied with monomers and vibrational energy and the corral keeps the latter from escaping. This demonstrates the coupling of the polymerizing function of a synthetic enzyme with processional movement.

RESULTS AND DISCUSSION

Both models demonstrate processional movement as a product of thermal capture: the ability of some kind of Brownian ratchet to channel certain vectorial vibrations but discarding other ones. This mechanism is in stark contrast to the one that is almost universally used to describe or understand molecular motors, one in which a source of chemical energy is used to exert a force through a fulcrum.

Both models are also useful in helping the students to visualize the modular construction of an enzyme. For example, students can easily equate the lengths of copper wire with peptide polymers, and the bending of the copper wire into unique shapes emphasizes and exemplifies the importance of tertiary and quaternary structure towards conferring functionality upon an otherwise-inert peptide polymer. The copper hairpins, which served as legs in these instructional models, might represent peptides, which at one point in their evolutionary history played an entirely different role but were then conscripted to serve as the feet of the molecular motor.

The models also illustrate quite effectively how even small genetic mutations can lead to entirely new functions. The very slight backward bending of the feet of teaching aid 1, reminiscent of a single amino acid substitution that now provides a novel kink in the molecular motor, allows the motor to operate in reverse relative to the parent molecule.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


