A major question for biomolecular physics has been “how do molecular motors use chemical energy to drive directed motion and the performance of mechanical work?” Aided by insights from the field of synthetic molecular machines, a remarkably simple and universal answer to this question can now be given - biomolecular machines carry out their function by diffusion on a sculpted energy landscape (R. D. Astumian et. al., Chem. Phys. Chem 17, 1719, 2016). An example landscape for the F1 subunit of the FoF1 ATP synthase (S. Mukherjee et. al., PNAS (USA) 108, 20550, 2011 ; S. Mukherjee et. al., Photosynth. Res. 134, 1, 2017) is shown in Fig. 1a. The structure (J. P. Abrahams et. al., Nature 370, 621, 1994) of the FoF1 ATP synthase is shown in Fig. 1b). Physiologically the Fo part of the molecule is embedded in the mitochondrial membrane. Proton flow induced by the proton motive force generated by the electron transport chain causes the c-ring to rotate, in turn causing the central stalk to rotate. This rotation is coupled to the synthesis of ATP. The F1 part of the motor (Fig. 1c)) can be isolated and immobilized on a surface. In this circumstance, hydrolysis of ATP catalyzed by the three subunits of the catalytic crown (stator) is coupled to rotation of the central stalk in the opposite direction to that driven by proton transport in intact mitochondria. The energy landscape in Fig. 1 a) is based on the structure of the F1 molecule where the chemical binding of the three subunits (E = empty, T = ATP, and D = ADP) and the relative angle of the central stalk are fixed and the system is allowed to relax to its minimum energy subject to those constraints, and that energy is plotted. The energies at “intermediate” chemical states are interpolated from these values.

The most obvious feature of the energy landscape is the pronounced blue and green zigzag valley connecting the upper left and lower right corners. There is no "non-equilibrium" character of this plot. If the system starts in the center (white circle) diffusion will bring the system to the lower right corner, a trajectory in which an ATP is hydrolyzed and the stalk completes a clockwise rotation, or lower left, in which an ATP is synthesized and the stalk completes a counter-clockwise rotation. These trajectories, labelled $\mathcal{F}$ and $\mathcal{F}^\dagger$, respectively, are much more likely than trajectories taking the system to the upper right, in which an ATP is synthesized and the stalk completes a clockwise rotation, or lower left, in which an ATP is hydrolyzed and the

Figure | Energy lands-cape and structures for the mitochondrial FoF1 ATP synthase. (a) Plots of the energy of the F1 molecule - a trimer of dimers plus a central stalk - subject to having the rotational angle of the central stalk and the chemical state of each of the three dimers constrained. (b) Structure of the complete FoF1 ATP synthase. (c) Ribbon structure of the trimer and the central stalk.
stalk completes a counter-clockwise rotation, labelled $B^\dagger$ and $B$, respectively. This symmetry breaking arises from the structure of the molecule, and the key feature is the relative heights of the barriers, with the lower barrier for the $F/F^\dagger$ trajectories labelled $\epsilon_F$ and the higher barrier for the $B/B^\dagger$ trajectories labelled $\epsilon_B$. (R. D. Astumian et al., Chem. Phys. Chem. 17, 1719, 2016; R. D. Astumian, Biophys J. 98, 2401, 2010; C. Pezato et al., Chem. Soc. Rev. 46, 5491, 2017).

So how is the symmetry between the $F$ and $F^\dagger$ trajectories to favor clockwise rotation (and between the $B$ and $B^\dagger$ trajectories) broken? The answer is simple - "mass action" - the principle according to which addition of substrate leads to creation of product, known also in chemistry as Le Chatlier’s principle. In our case, having more than the equilibrium amount of ATP leads to hydrolysis of ATP to form ADP and Pi, i.e., to motion in the downward direction on the energy landscape in Fig. 1a). Because of the structure-based sculpting of this landscape with appropriately located barriers, the downward tendency is inexorably accompanied by motion to the right (i.e., by clockwise rotation).

This answer - that directed motion results from diffusion - is unsatisfying to many biophysicists who prefer to think in terms of mechanically based descriptions in which input energy is used to promote a molecular machine to a high-energy intermediate state from which directional relaxation in a "power-stroke" is accompanied by "force" or "torque" generation allowing the motor to perform mechanical work (W. Hwang et al., PNAS (USA) 116, 19777, 2019). These mechanical pictures of molecular machines are solidified by experiments on synthetic light-driven motors or motors driven by externally enforced changes in the environment. Further, artistic conceptualization embodying these mechanical ideas, are widely available as movies on the web. Beautiful and inspiring though they may be, such animations are intrinsically misleading. Part of the problem is that light driven synthetic motors operate exactly as has been described in textbooks since the 1950’s, a seeming resounding confirmation of these ideas involving a power-stroke.

This is not the case - photochemical excitation does not obey the principle of microscopic reversibility, a principle that governs all thermally driven processes including catalysis of chemical reactions (R. D. Astumian, Faraday Discuss. 195, 583 (2016). Thus, the "power-stroke" that is the key factor determining directionality of light driven motors is utterly irrelevant (R. D. Astumian, Biophys. J. 108, 291, 2015) for determining the directionality and strength of motors driven by hydrolysis of ATP or by transport of ions across a membrane. This is beautifully illustrated in recent work on Myosin by the Warshel group (S. Mukherjee et. al., PNAS (USA) 114, 2259, 2017 ; R. Alhadeff, et. al., PNAS (USA) 114, 10426, 2017).

To better understand these points let us consider an F1 ATPase at chemical equilibrium with equal chemical potentials of ATP, ADP, and inorganic phosphate. Then in our thought experiment we can move away from equilibrium by adding ATP to the solution.

Unlike mechanical equilibrium, chemical equilibrium is dynamic, with constant motion due to thermal noise i.e, collision between the molecular machine and solvent molecules. Consequently, an F1 molecule diffusing on the energy surface shown in Fig. 1a) is all the time moving, sometimes completing a trajectory toward the lower right-hand corner, but with equal likelihood completing a trajectory toward the upper left-hand corner. Occasionally, but with much lower probability, the F1 will complete a trajectory to the lower left-hand corner, and with equally low probability, to the upper right-hand corner. This is the dynamic equilibrium in which there is constant motion, but where each trajectory is exactly as likely as its microscopic reverse. When we add ATP to bring the system out of thermodynamic equilibrium, what happens? Do the ATP molecules now violently kick the F1 when they bind and are hydrolyzed? Does the conformational diffusion of the F1 lead to some power-stroke or judo throw? Does the presence of ATP in excess of its equilibrium lead to any change in the character of the trajectories by which rotation occurs such that even in principle there could be a structural "smoking gun" to which one could point and say "Aha, there is the origin of the directionality!" The answer is NO! The only change when we add ATP is that those trajectories in which ATP binds and is hydrolyzed become more likely than their microscopic reverses in which ADP and Pi bind and are synthesized to form ATP. Catalysis driven molecular machines work by diffusion on a sculpted energy landscape where the disequilibrium in the catalyzed reaction favors the functional motion by mass action.

**Review Editor’s Views**  This is an excellent paper that examines the origin of the catalytic motion in biological machines. The paper presents very convincing evidence that the motion is diffusive in nature and thus cannot be described by an inertial or by a power stroke model. This work will be of enormous interest to the readers.