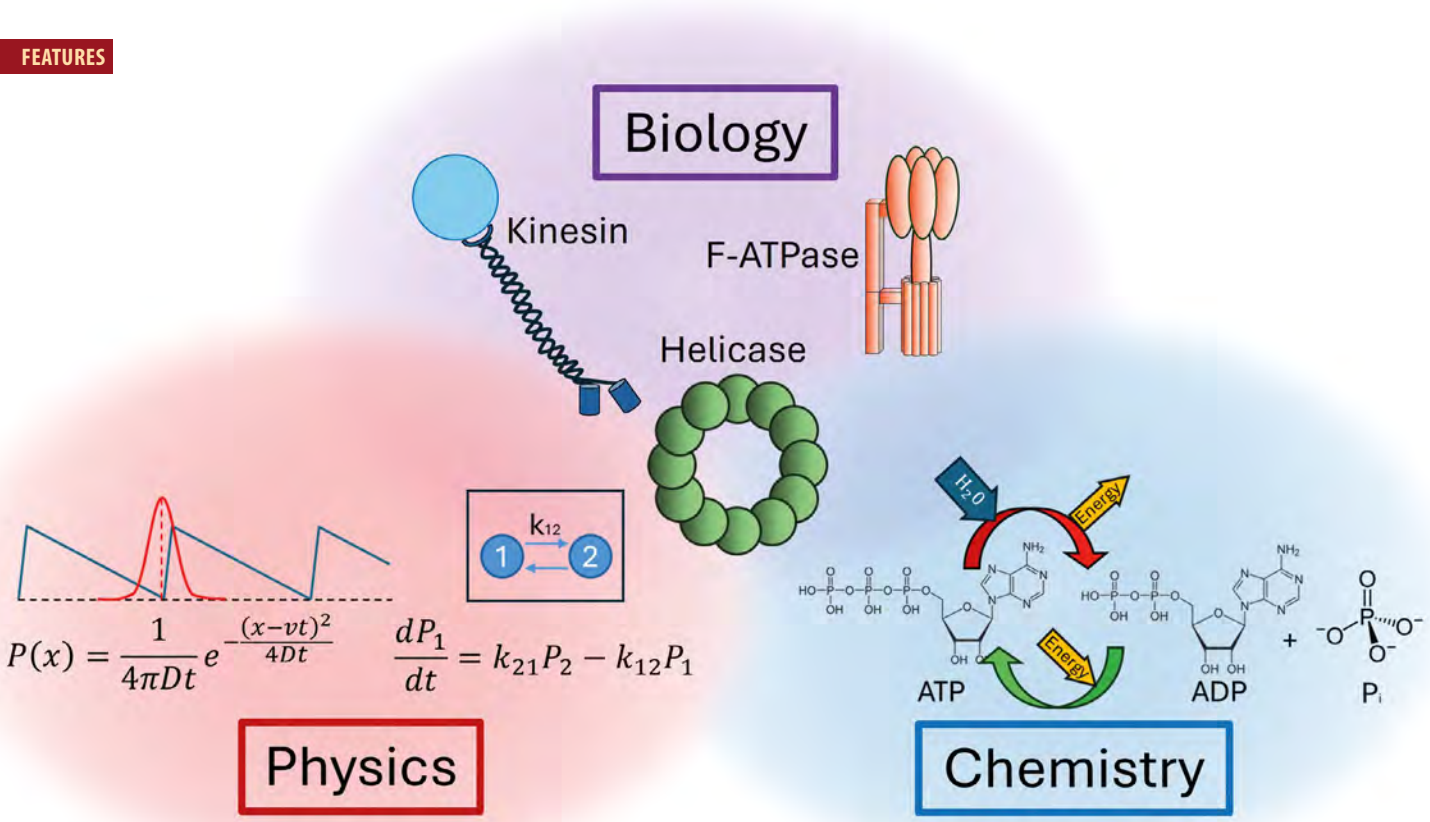

CONTROLLED TRANSPORT BY MOLECULAR MACHINES: EXPLORING BIOLOGICAL MOTORS AND THEIR PHYSICS

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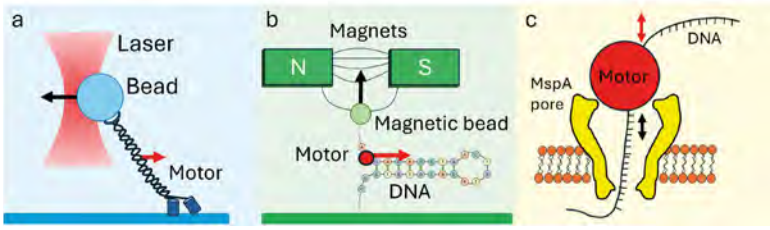
Molecular motors are fascinating biological machines that play a crucial role in a variety of cellular processes, including mass transport, muscle contraction, DNA replication, transcription and repair, and RNA translation. These structures convert chemical energy from adenosine triphosphate (ATP) hydrolysis into mechanical work.

ATP serves as the primary currency in the chemical reactions occurring in biological systems. ATP hydrolysis involves the breaking of a high-energy phosphate bond within ATP, resulting in the formation of ADP (adenosine diphosphate) and inorganic phosphate (Pi). This process releases energy on the order of 10-20 kBT (where kB is the Boltzmann constant and T is the temperature). At room temperature, the thermal agitation energy equals $1 \cdot k_BT = 4 \cdot 10^{-21}$J=4pN·nm (1pN=1picoNewton=10^{-12}N and 1nm=1nanometer=10^{-9}m). Molecular motors operate at the nanoscale in highly noisy and viscous environments against forces in the pN scale.

Despite these conditions, some molecular motors exhibit surprisingly high efficiencies. Particularly interesting is the case of F1-ATPase, an enzyme responsible for generating ATP from ADP and inorganic phosphate, which operates with efficiency close to 100%. Researchers have long been intrigued by the question of how biological molecular motors can function so effectively in highly noisy environments [1].

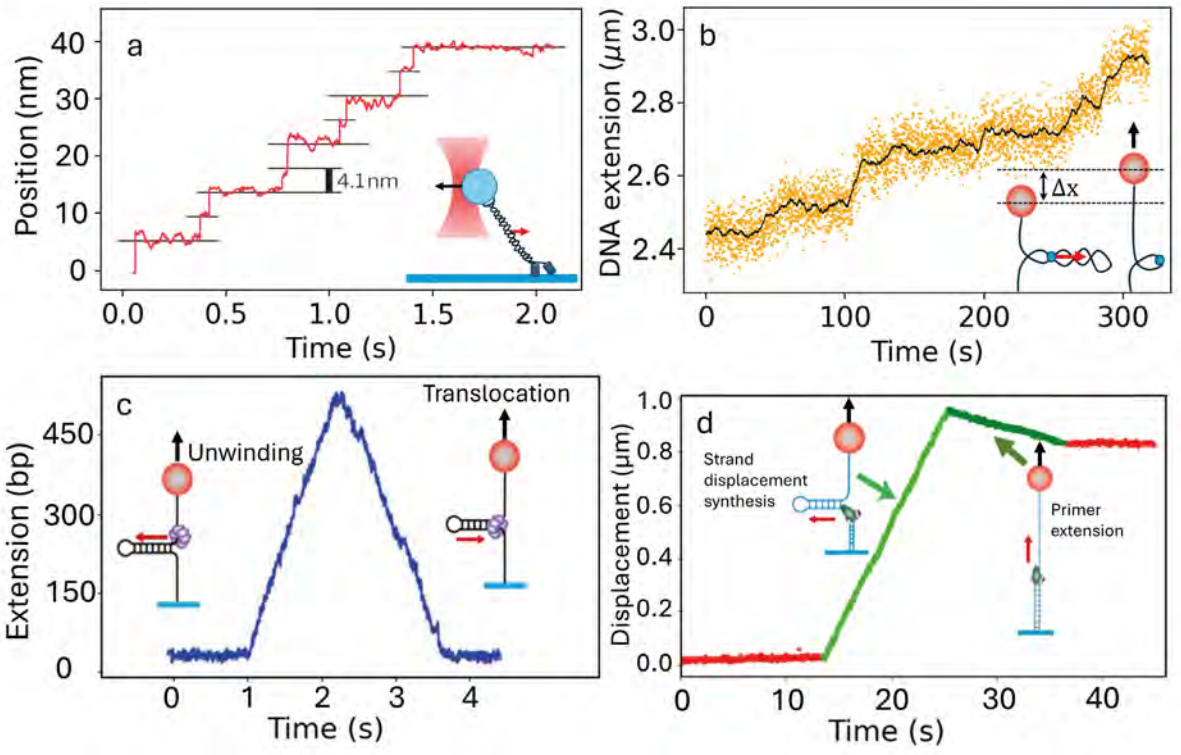
Traditionally, molecular motors have been studied using standard biochemical ensemble assays that measure the average behaviour of a large ensemble of molecules. Average measurements provide limited information about rare events and heterogeneous dynamics. Single-molecule techniques have remodelled the field of molecular biophysics, permitting access to monitoring individual reaction coordinates informative of molecular physico-chemical properties such as conformation, orientation, and position [2,3].



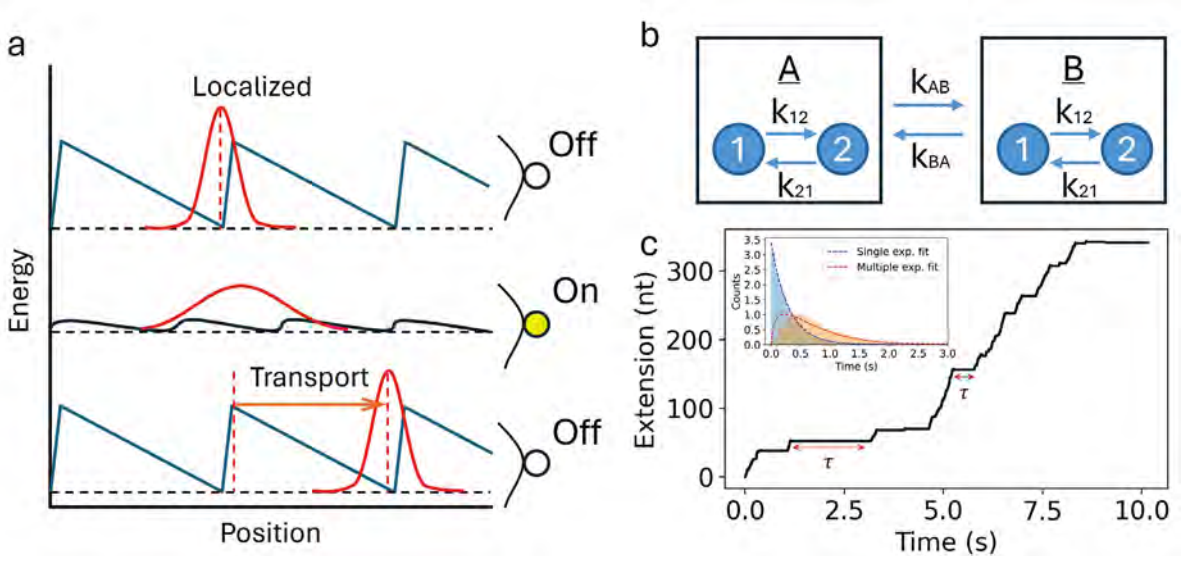
▲ **FIG. 1:** Schematic representation of SME. a) LOT experiment showing an optically trapped bead tethered to a kinesin motor. b) MT experiments showing the magnets pulling on a DNA hairpin to study a DNA translocating motor (red circle). c) SNPRT experiment showing a biological nanopore inserted in a lipid bilayer and a DNA molecule whose translocation is controlled by a motor. Force is controlled by applying a voltage difference across the membrane. Black and red arrows indicate the direction of applied force and the motor direction respectively.

Techniques

Structural information about these motors emerges through various microscopy and spectroscopy techniques. Examples are X-ray crystallography which uses the unique pattern of scattered X-rays by the crystallized sample, or cryo-electron microscopy which studies biological samples by rapidly freezing them to preserve their natural state. Structural techniques are complemented by single-molecule experiments (SME) that monitor the dynamic behaviour of individual motors with high spatial and temporal resolution. SME include fluorescence and force spectroscopy. They have been combined in the lab, and in commercial instruments making SME accessible to biology research groups worldwide. Fluorescence techniques are total internal reflection fluorescence (TIRF) for single-molecule localization and Förster resonance energy transfer (FRET) for conformational dynamics, among others [4]. Force spectroscopy techniques permit the direct manipulation by exerting forces on single molecules [5]. Examples are atomic force microscope (AFM), laser optical tweezers (LOT), magnetic tweezers (MT), and acoustic force-spectroscopy (AFS), among others. Mechanical manipulation is achieved by tethering a single molecule between a surface and a force probe (cantilever tip or optically/magnetically trapped bead). With them, we can controllably exert mechanical forces and torques while measuring the molecular extension. AFM and LOT are suitable to study cellular and molecular interactions by exerting forces in the pN to nN range (1nN=1nanoNewton=10-9N) with sub-nanometer spatial resolution over timescales limited by the corner frequency of the probe, typically in the range 1-100kHz. MT can detect forces ranging from fN (1fN=1femtoNewton=10^{-15}N) to ~100 pN, with a temporal resolution limited by the imaging frequency acquisition. Compared to AFM, LOT and MTs can exert torque, proving them very useful in studying topoisomerases, which are responsible for relieving torsional stress created in DNA during polymerase transcription and replication. Furthermore, MTs are a ● ● ●



◀ **FIG. 2:** SME traces for different motors. **a)** Kinesin walking on a microtubule studied with LOT. **b)** Topoisomerase unwinding a DNA hairpin using MT. **c)** Helicase unwinding a DNA hairpin using MT, the rising edge corresponds to the DNA unwinding catalysed by the helicase, whereas the falling edge corresponds to the helicase translocation while the DNA reforms in its wake. **d)** Polymerase copying a DNA hairpin using MT, while it opens the DNA. Black and red arrows indicate the direction of the applied force and the motor direction respectively.



▲ **FIG. 3: a)** Ratchet model described by an energy landscape that changes depending on the ATP cycle state (On/Off). The ATP hydrolysis changes the energy landscape in a way that favours the movement in a specific direction. **b)** Discrete model including two main states (A and B) with different sub-states (1 and 2), whose transitions are characterized by kinetic rates **c)** Simulated trace of a molecular motor following the scheme depicted in panel (b). Two lifetime events are shown in red arrows as an example. Inset: Comparison between lifetime distributions for a single-step reaction (single exponential) and multiple-step reaction (multiple exponential).

high-throughput technique with the capability of parallel measurements by the simultaneous tracking of multiple molecular tethers in a single experiment.

Recent methods include electrical measurements of single motor enzymes translocating nucleic acids through solid-state and biological pores. Jens Gundlach and coworkers, from the University of Washington in Seattle, introduced Single-molecule Picometer Resolution Nanopore Tweezers (SPRNT), a technique that provides readout of a DNA sequence moving through an enzyme, allowing to investigate sequence effects on the enzymatic activity with high spatiotemporal resolution [6].

Motors

Kinesin and myosin were the first studied molecular motors using optical traps [7]. Kinesin is a dimeric and processive motor that moves along a microtubule through a coordinated action of its two heads, playing a central role in cellular transport of vesicles. Myosin is a non-processive motor responsible for muscle contraction by pulling on actin filaments. Myosin works cooperatively in concerted action with other myosins. Other examples of processive motors include DNA and RNA translocating motors such as helicases and polymerases [8] which are involved in DNA replication, transcription, recombination, and repair. Helicases promote DNA unwinding, whereas polymerases catalyse new DNA strands.

Theoretical models for processive enzymes

Theoretical modelling aims at capturing the mechanochemical cycle of the enzyme relating ATP hydrolysis to its movement. We can differentiate two types of models: continuum ratchet models and discrete Markov chains [9]. In continuum models the motor diffuses over an asymmetric energy landscape characterized by energy barriers and wells. The jumps over the barriers are thermal activated processes and the ATP hydrolysis can modify the landscape lowering the barriers and promoting the forward motion. The landscape asymmetry favours forward direction (as compared to backward motion) generating directed motion. In discrete models, the motor moves from state A to state B through several biochemical sub-states. In these substates the motor can undergo conformational changes or chemical reactions such as ATP hydrolysis.

A relevant kinetic descriptor is the first passage time or lifetime of the motor defined as the average amount of time spent in each state. If the motor's cycle has a single rate-limiting step, the lifetime distribution is a Poisson process, whereas in the presence of multiple steps, the distribution is more complex. For some kinesins lifetimes are exponentially distributed [7], suggesting that one ATP molecule is hydrolysed at each step. In contrast, some helicases exhibit multi-exponential lifetime distributions indicating the presence of several intermediates, such as backward steps [6]. It is an open question whether such backwards steps consume steps or rather re-synthesize ATP from ADP and Pi.

A fundamental measurement to characterize the relationship between the biochemical and mechanical cycle of the motor is the measurement of the fundamental step. This has been achieved for various motors, such as kinesin with an 8nm step, F1-ATPase with 90° rotation steps and helicases and polymerases with one base pair step (0.34nm). With the development of high-resolution setups, it has been possible to observe that some motors exhibit sub-steps such as kinesin with 4nm sub-steps [10] ●●●

and F1-ATP-ase with 30º sub-steps [11]. Finally, even half-base pair sub-steps have been observed for a viral helicase [12].

Molecular motors, the machines of life, are a paradigm for mass, energy, and information transport processes in the cell. The possibility of manipulating and monitoring them with nanometric precision and accuracy permits biologists to investigate such processes with unprecedented detail. At the same time, physicists can investigate and test fundamental theories for the behavior of living matter. ■

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