Machines of life: catalogue, stochastic process modeling, probabilistic reverse engineering and the PIs- from Aristotle to Alberts

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Molecular machines consist of either a single protein or a macromolecular complex composed of protein and RNA molecules. Just like their macroscopic counterparts, each of these nano-machines has an engine that “transduces” input energy into an output form which is then utilized by its coupling to a transmission system for appropriate operations. The theory of heat engines, pioneered by Carnot, rests on the second law of equilibrium thermodynamics. However, the engines of molecular machines, operate under isothermal conditions far from thermodynamic equilibrium. Moreover, one of the possible mechanisms of energy transduction, popularized by Feynman and called Brownian ratchet, does not even have any macroscopic counterpart. But, molecular machine is not synonymous with Brownian ratchet; a large number of molecular machines actually execute a noisy power stroke, rather than operating as Brownian ratchet. The man-machine analogy, a topic of intense philosophical debate in which many leading philosophers like Aristotle and Descartes participated, was extended to similar analogies at the cellular and subcellular levels after the invention of optical microscope. The idea of molecular machine, pioneered by Marcelo Malpighi, has been pursued vigorously in the last fifty years. It has become a well established topic of current interdisciplinary research as evident from the publication of a very influential paper by Alberts towards the end of the twentieth century. Here we give a non-technical overview of the strategies for (a) stochastic modeling of mechano-chemical kinetic processes, and (b) model selection based on statistical inference drawn from analysis of experimental data. It is written for non-experts and from a broad perspective, showing overlapping concepts from several different branches of physics and from other areas of science and technology.

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I. INTRODUCTION

Cell is the structural and functional units of life. How “active” is the interior of a living cell? Imagine an under water “metro city” which is, however, only about 10 µm long in each direction! In this city, there are “highways” and “railroad” tracks on which motorized “vehicles” transport cargo to various destinations. It has an elaborate mechanism of preserving the integrity of the chemically encoded blueprint of the construction and maintenance of the city. The “factories” not only supply their products for the construction and repair works, but also manufacture the components of the machines. This eco-friendly city re-charges spent “chemical fuel” in uniquely designed “power plants”. This city also uses a few “alternative energy” sources in some operations. Finally, it has special “waste-disposal plants” which degrade waste into products that are recycled as raw materials for fresh synthesis. All the automated processes in this high-tech micro-city are run by nano-machines. This is not the plot of a science fiction, but a dramatized picture of the dynamic interior of a cell.

A molecular machine is either a single protein or a macromolecular complex consisting of proteins and RNA molecules. Just like their macroscopic counterparts, these nano-machines take an input (most often, chemical energy) and it transduces input energy into an output. If the output is mechanical work the machine is usually referred to as a molecular motor. Similarly, a cell can also be regarded as a micron-size “energy transforming device”. The concept of “machine” is not restricted only to subcellular or cellular levels of biological organization. In ancient times, philosophers proposed the concept of “living machine” to describe a whole animal, including a
man; we’ll discuss the evolution of this concept towards the end of this article.

Not only animals, but even plants also move in response to external stimuli and movements take place in plants at all levels—from whole plant and plant cell to the subcellular level. I cannot resist the temptation of quoting Thomas Huxley’s poetic description of cytoplasmic streaming, in particular, and of intracellular motor traffic, in general, in plants: “...the wonderful noonday silence of a tropical forest is, after all, due only to the dulness of our hearing; and could our ears catch the murmur of these tiny maelstroms, as they whirl in the innumerable myriads of living cells which constitute each tree, we should be stunned, as with the roar of a great city”.

In this article, however, we focus attention almost exclusively on molecular machines that drive subcellular processes within living cells. The processes driven by molecular machines include not only intracellular motor transport, but also manipulation, polymerization and degradation of the bio-molecules. Explaining the physical principles that govern these machine-driven processes will bring us closer to the ultimate goal of biological physics: understanding “what is life” in terms of the laws of physics (and chemistry).

Biomolecular machines operate in a domain where the appropriate units of length, time, force and energy are, nano-meter, milli-second, pico-Newton and $k_B T$, respectively ($k_B$ being the Boltzmann constant and $T$ is the absolute temperature). Aren’t the operational mechanism of molecular machines similar to their macroscopic counterparts except, perhaps, the difference of scale? NO. In spite of the striking similarities, it is the differences between molecular machines and their macroscopic counterparts that makes the studies of these systems so interesting from the perspective of physicists.

This article is a brief guide for a beginner embarking on an exploration of the exciting frontier of research on molecular machines. It begins with a catalog of the known molecular machines and a list of fundamental questions on their operational mechanism that the explorer should try to address. Like any travel guide, this article also cautions the explorer about the counter-intuitive phenomena that await him/her in this new territory of his/her exploration where he/she may need new sophisticated technical tools that were not needed for handling macroscopic machines. For the explorer, this article also charts a tentative road map along with a summary of the mathematical strategies that he/she might use to make progress in this frontier territory. Excursions to some of the listed border areas that overlap with areas of research in other branches of science and engineering could be enjoyable and intellectually rewarding for the explorer. For any explorer, it is good to know the experience of the pioneers. The final section, on the evolution of the concept of biological machines, is likely to be enjoyed as a dessert by physicists, and as a fresh food for thought by historians and philosophers of science.

II. A CATALOGUE: TYPICAL EXAMPLES OF MOLECULAR MACHINES AND FUELS

Based on the mode of operation, the biomolecular machines can be divided broadly into two groups. Cyclic machines operate in repetitive cycles in a manner that is very similar to that of the cyclic engines which run our cars. In contrast, some other molecular machines are one-shot machines that exhaust an internal source of free energy in a single round. Force exerted by a compressed spring, upon its release, is a typical example of a one-shot machine.

A. Fuels for molecular machines

The most common way of supplying energy to a natural nanomachine is to utilize the chemical energy (or, more appropriately, free energy) released by a chemical reaction. Most of the machines use the so-called high-energy compounds—particularly, nucleoside triphosphates (NTPs)—as an energy source to generate the mechanical energy required for their directed movement. The most common chemical reaction is the hydrolysis of ATP (ADP): $ATP \rightarrow ADP + P_i$. Some other high-energy compounds can also supply input energy; one typical example being the hydrolysis of Guanosine Triphosphate (GTP) to Guanosine Diphosphate (GDP). Under normal physiological conditions, hydrolysis of ATP is extremely slow. Most of the molecular motors fuelled by ATP function also as ATPase enzyme (i.e., catalyzes hydrolysis of ATP) thereby speeding up the energy-supplying reaction by several orders of magnitude.

If a cyclic machine runs on a specific chemical fuel then the spent fuel must be removed as waste products and fresh fuel must be supplied to the machine. Fortunately, normal cells have machineries for recycling waste products to manufacture fresh fuel, e.g., synthesizing ATP from ADP. This raises an important question: since ATP is a higher-energy compound than ADP, how are the normal cells able to manufacture fresh fuel, e.g., synthesizing ATP from ADP? This raises an important question: since ATP is a higher-energy compound than ADP, how are the ATP-synthesizing machines driven to perform this energetically “uphill” task? Fortunately, chemical fuel is not the only means by which input energy can be supplied to intracellular molecular machines.

A cell gets its energy from external sources. It has special machines to convert the input energy into some “energy currency”. For example, chemical energy supplied by the food we consume is converted into an electro-chemical potential $\Delta \mu$ that not only can be used to synthesize ATP, but can also directly run some other machines. In plants similar proton-motive forces are generated by machines which are driven by the input sunlight. Only hydrogen ion ($H^+$), i.e., a proton, and sodium ion ($Na^+$) are used in the kingdom of life to create the electro-chemical potentials, i.e., it used either a proton-motive force (PMF) or a sodium-motive force (SMF) as an energy currency.

The advantages of using light, instead of chemical re-
TABLE I: Superfamilies of motor proteins and the corresponding tracks.

| Motor superfamily | Filamentous track | Minimum step size |
|-------------------|-------------------|-------------------|
| Myosin            | F-actin           | 36 nm             |
| Kinesin           | Microtubule       | 8 nm              |
| Dynein            | Microtubule       | 8 nm              |

action, as the input energy for a molecular motor are as follows: (i) light can be switched on and off easily and rapidly, (ii) usually, no waste product, which would require disposal or recycling, is generated.

Thus, study of molecular machines deals with two complementary aspects of bioenergetics: (a) conversion of energy input from the external sources into the energy currency of the cell, and (b) conversion of the energy currency to drive various active other active processes.

B. Cytoskeletal motors

The cytoskeleton of a cell is the analogue of the human skeleton [3]. However, it not only provides mechanical strength to the cell, but its filamentous proteins also form the networks of “highways” (or, “tracks”) on which cytoskeletal motor proteins [3, 4] can move. Filamentous actin (F-actin) and microtubules (MT) which serve as tracks are “polar” in the sense that the structure and kinetics of the two ends of each filament are dissimilar.

The superfamilies of cytoskeletal motors and the corresponding filamentous tracks are listed in table I. Every superfamily can be further divided into families. Members of every family move always in a particular direction on its track; for example, kinesin-1 and cytoplasmic dynein move towards + and - end of MT, respectively. Similarly, myosin-V and myosin-VI move towards the + and - ends of F-actin, respectively.

For their operation, each motor must have a track-binding site and another site that binds and hydrolyzes ATP (so-called ATPase site). Both these sites are located, for example, in the head domain of myosins and kinesins both of which walk on their heads! The motor-binding sites on the tracks are equispaced; the actual step size of a motor can be, in principle, an integral multiple of the minimum step size which is the separation between two neighboring motor-binding sites on the corresponding track.

1. Porters

Some linear motors are cargo transporters [12]; however, the size of the cargoes are usually much larger than the motor itself! It is desirable that such a motor “walks” for a significant distance on its track carrying the cargo; for obvious reasons, such motors are referred to as porters [13]. Kinesin and dyneins attached simultaneously to the same “hard” cargo can get engaged in a tug-of-war leading to a bidirectional movement of the cargo [14, 15]. In regions of overlap between MT and F-actin filaments, a large cargo may be hauled simultaneously by kinesins and myosins which, however, walk on their respective tracks. Alternatively, along its journey route, a cargo may be transferred from the MT-based transport network, which dominate at the cell center, to the F-actin based network that covers most of the cell periphery [16]. A “soft” cargo pulled by many kinesins can get elongated into a tube [17].

2. Machines for chipping filamentous tracks

A MT depolymerase is a kinesin motor that chips away its own track from one end [18]. Members of the kinesin-13 family can reach either end of the MT diffusively (without ATP hydrolysis) and, then, start chipping the track from the end where it reaches. In contrast, members of the kinesin-8 family walk towards the plus end of the MT track hydrolyzing ATP and after reaching that end starts chipping it from there [19, 20]. Chipping by both families of depolymerase kinesins are energized by ATP hydrolysis.

3. Contractility: motor-filament crossbridge and collective dynamics of sliders and rowers

Some motors are capable of sliding two different filaments with respect to each other by stepping simultaneously on these two filaments [21]. Some sliders work in groups and each detaches from the filament after every single stroke; these are often referred to as rowers because of the analogy with rowing with oars [13]. Contractility, rather than motility, at the subcellular and cellular level are driven by the sliders and rowers [22, 23]. Some examples of this category are listed in the table II, the details can be found in the cited review articles.
| Motor | Sliding filaments | Function (example) |
|-------|-------------------|--------------------|
| Myosin | “Thin filaments” (F-actin) of muscle fibers | Muscle contraction [24] |
| Myosin | “Stress fibers” (F-actin) of non-muscle cells | Cell contraction [25] |
| Myosin | Cytokinetic “contractile ring” (F-actin) in eukaryotes | Cell division [26] |
| Kinesin | Interpolar microtubules in mitotic spindle | Mitosis [27, 28] |
| Dynein | Microtubules of axoneme | Beating of eukaryotic flagella [29, 30] |
| Dynein | Microtubules of megakaryocytes | Blood platelet formation [31] |

**TABLE II:** Few example of cytoskeletal rowers and sliders as well as their biological functions.

| Polymer | mode of force generation | Function (example) |
|---------|--------------------------|--------------------|
| MT      | polymerizing piston-like  | organizing cell interior [35] |
| F-actin | polymerization            | cell motility [36] |
| FtsZ    | polymerization            | bacterial cytokinesis [37] |
| MSP     | polymerization            | motility of nematode sperm cells [38] |
| Type-IV pil | de-polymerization       | Eukaryotic chromosome segregation [33] |
| MT      | de-polymerization         | vorticellid spasmoneme [34] |
| sparsmin | spring-like               | egg fertilization by sperm cell of the horse-shoe crab Limulus polyphemus [40] |
| Coiled actin | spring-like              | |

**TABLE III:** Force generation by polymerizing/depolymerizing, coiling/uncoiling filaments: pistons, hooks and springs.

4. Push and pull of cytoskeletal filaments: nano-pistons and nano-springs

Elongation of filamentous biopolymers that presses against a light object (e.g., a membrane) can result in a “push” [32]. Similarly, a depolymerizing tubular filament can “pull” a light ring-like object by inserting its hook-like outwardly curled depolymerizing tip into the ring [33]. A flexible filament, upon compression by input energy, can store energy that can perform mechanical work when the filament springs back to its original relaxed shape [34]. Some typical examples are given in table III.

C. Machines for synthesis, manipulation and degradation of macromolecules of life

1. Membrane-associated machines for macromolecule translocation: exporters, importers and packers

In many situations, the motor remains immobile and pulls a macromolecule; the latter are often called translocase. Some translocases export (or, import) either a protein [41] or a nucleic acid strand [42, 43] across the plasma membrane of the cell or, in case of eukaryotes, across internal membranes. A list is provided in table IV.

The genome of many viruses are packaged into a prefabricated empty container, called viral capsule, by a powerful motor attached to the entrance of the capsule. As the capsule gets filled, the pressure inside the capsule increases which opposes further filling [44, 45]. The effective force, which opposes packaging, gets contributions from three sources: (a) bending of stiff DNA molecule inside the capsid; (b) strong electrostatic repulsion between the negatively charged strands of the DNA; (c) loss of entropy caused by the packaging [46]. The packaging motor has to be powerful enough to overcome such a high pressure.

2. Machines for degrading macromolecules of life

Restriction-modification (RM) enzyme defend bacterial hosts against bacteriophage infection by cleaving the phage genome while the DNA of the host bacteria are not cleaved. Exosome and proteasome are machines that shred RNA and proteins into their basic subunits, namely, nucleotides and amino acids, respectively. Similarly, there are machines for degrading polysachharides, e.g., cellulose (a cellulose degrading machine), starch degrading enzymes, chitin degrading enzyme (chitinase), etc. These machines are listed in table V.

| Membrane | Polymer |
|----------|---------|
| Nuclear envelope | RNA/Protein [48, 47] |
| Membrane of endoplasmic reticulum | Protein [48] |
| Membranes of mitochondria/chloroplasts | Protein [49, 50] |
| Membrane of peroxisome | Protein [51] |

**TABLE IV:** Membrane-bound translocases.
3. Machines for template-dictated polymerization

Two classes of biopolymers, namely, polynucleotides and polypeptides perform wide range of important functions in a living cell. DNA and RNA are examples of polynucleotides while proteins are polypeptides. Both polynucleotides and polypeptides are made from a limited number of different species of monomeric building blocks, namely, nucleotides and amino acids, respectively. The sequence of the monomeric subunits to be used for synthesis of each of these are dictated by that of the corresponding template. These polymers are elongated, step-by-step, during their birth by successive addition of monomers, one at a time. The template itself also serves as the track for the polymerizer machine that takes chemical energy as input to polymerize the biopolymer as well as for its own forward movement. Therefore, these machines are also referred to as motors.

Depending on the nature of the template and product nucleic acid strands, polymerases can be classified as DNA-dependent DNA polymerase (DdDP), DNA-dependent RNA polymerase (DdRP), etc. as listed in the table VI.

### TABLE V: Machines for degradation of macromolecules of life.

| Polymer          | Examples of Machines |
|------------------|----------------------|
| DNA (polynucleotide) | RM enzyme [52]       |
| RNA (polynucleotide) | Exosome [53]         |
| Protein (polypeptide) | Exosome [53]         |
| Cellulose (polysacharide) | Cellulase [56]      |
| Starch (polysacharide) | Starch degrad. enzyme [53] |
| Chitin (polysacharide) | Chitinase [57]      |

| Machine | Template | Product | Function |
|---------|----------|---------|----------|
| DdDP    | DNA      | DNA     | DNA replication [58] |
| DdRP    | DNA      | RNA     | Transcription [59–61] |
| RdDP    | RNA      | DNA     | Reverse transcription [62] |
| RdRP    | RNA      | RNA     | RNA replication [63] |
| Ribosome | mRNA | Protein | Translation [64–66] |

### TABLE VI: Types of polymerizing machines, the templates they use and the corresponding product of polymerization.

3. Machines for template-dictated polymerization

4. Unwrappers and unzippers of packaged DNA: chromatin remodelers and Helicases

In an eukaryotic cell DNA is packaged in a hierarchical structure called chromatin. In order to use a single strand of the DNA as a template for transcription or replication, it has to be unpackaged either locally or globally. ATP-dependent chromatin remodelers [67] are motors that perform this unpackaging. However, only one of the strands of the unpackaged duplex DNA serves as a template: the duplex DNA is unzipped by a DNA helicase [68, 69]. Similarly, a RNA helicase unwinds a RNA secondary structure. During DNA replication, a helicase moves ahead of the polymerase, like a mine sweeper, unzipping the duplex DNA and dislodging other DNA-bound proteins. However, the transcriptional and translational machineries do not need assistance of any helicase because these are capable of unzipping DNA and unwinding RNA, respectively, on their own. A helicase can be monomeric, or dimeric or hexameric.

### D. Rotary motors

Rotary molecular motors are, at least superficially, very similar to the motor of a hair dryer. Two rotary motors have been studied most extensively. (i) A rotary motor embedded in the membrane of bacteria drive the bacterial flagella [70, 71] which, the bacteria use for their swimming in aqueous media. (ii) A rotary motor, called ATP synthase [72, 73], is embedded on the membrane of mitochondria, the powerhouses of a cell. A synthase drives a chemical reaction, typically the synthesis of some product; the ATP synthase produces ATP, the “energy currency” of the cell, from ADP.

### E. Processivity, duty ratio, stall force and dwell time

One can define processivity of a motor in three different ways:

(i) Average number of chemical cycles in between attachment and the next detachment from the track;

(ii) mean time of a single run, i.e., in between an attachment and the next detachment of the motor from the track;

(iii) mean distance spanned by the motor on the track in a single run.

Since the definitions (ii) and (iii) are directly accessible to experiments, these are more useful than the definition (i). Another related, but distinct, concept is that of duty ratio which is defined as the average fraction of the time that each head spends remaining attached to its track during one cycle.

To translocate processively, a motor may utilize one of the three following strategies:

**Strategy I:** the motor may have more than one track-binding domain (oligomeric structure can give rise to such a possibility quite naturally). Most of the cytoskeletal motors like conventional two-headed kinesin use such a strategy [74]. One of the track-binding sites remains bound to the track while the other searches for its next binding site.

**Strategy II:** A motor may possess non-motor extra domains or some accessory protein(s) bound to it which can interact with the track even when none of the motor domains of the motor is attached to the track. Dynamin seems to exploit dynactin [75] to enhance its own processivity.
Strategy III: it can use a “clamp-like” device to remain attached to the track; opening of the clamp will be required before the motor detaches from the track. For example, DdDP utilizes this strategy. An external force directed opposite to the natural direction of walk of a motor is called a load force. Average velocity of a motor decreases with increasing load force. The magnitude of the load force at which the average velocity of the motor vanishes, is called the stall force. The force-velocity relation is one of the most fundamental characteristic properties of a motor. Its status in biophysics is comparable, for example, to that of the I-V characteristics of a device in semiconductor physics.

Two motors with identical average velocities may exhibit widely different types of fluctuations. Therefore, as we’ll show in detail, a deeper understanding of the operational mechanism of a motor can be gained from the distributions of their “dwell” at the successive spatial positions on the track.

F. Fundamental general questions

(i) Single-molecule mechanism: How do the interactions among the component structural units of an individual motor, motor-track interactions, motor-ligand (fuel) interactions and the mechano-chemical kinetics of the system determine, for example, (a) the directionality, (b) processivity, (c) dwell-time distribution, (d) force-velocity relation, and (e) efficiency of transduction? Does a given dimeric motor walk hand-over-hand or crawl like an inchworm, and why? Do the ATPase domains of a hexameric motor “fire” (a) in series, or (b) in parallel, or, (c) in random sequence?

(ii) Multi-motor coordination in a “workshop”: The motors do not work in isolation in-vivo. What are the mechanisms and consequences of the spatio-temporal coordination of the motors? For example, a single mitotic spindle consists of many polymerizing and depolymerizing MTs, several types of cytoskeletal motors, including depolymerases. A replisome, the workshop for DNA replication, has to coordinate the operations of clamps and clamp loaders with those of primases, polymerases, etc. Similarly, a ribosome is a mobile platform on which the operations of several devices have to be coordinated properly during protein synthesis. Well known co-directional as well as head-on collisions of polymerases and those of ribosomes can create traffic jam under some circumstances whereas a collision can restart stalled traffic in other circumstances.

III. MOTORING IN A VISCOS FLUID: FROM NEWTON TO LANGEVIN

Force is one of the most fundamental quantities in physics. As we’ll argue in this section, some of the forces which dominate the dynamics of molecular machines have negligible effect on macroscopic machines.

A. Newton’s equation: deterministic dynamics

For simplicity, let us first consider a hypothetical scenario where neither the tracks nor the fuel molecules are present in the aqueous medium in which there is a free (i.e., not bound to any other molecule) motor protein. Suppose the medium consists of only N “particle-like” molecules and the center of mass of the motor protein is also represented by a “particle”. Then, the exact trajectories of all these N + 1 “particles” can be obtained by solving the corresponding coupled Newton’s equations that describe the dynamics of the N + 1 particles. In reality, this approach is impractical because, even with the largest and fastest computers available at present, we cannot get the trajectories over time intervals of the order of Is which is relevant for majority of the motor proteins as long as N is large (N ~ 10^{23}).

B. Langevin equation: stochastic dynamics

A more pragmatic approach would be to solve only the equations of motion for the motor protein by treating the N particles of the medium as merely the constituents of a reservoir. In other words, one monitors the motion in a 6-dimensional subspace of the full 6(N + 1)-dimensional phase space of the system. The price one has to pay for this simplification is that instead of the original Newton’s equation for the motor protein, one has to now solve a Langevin equation that describes the “Brownian” motion of the motor protein. Since we are soon going to extend the discussion in the presence of filamentous tracks which are essentially one-dimensional, we write the Langevin equation for the “Brownian” motion of the motor protein in one-dimension m(d^2x/dt^2) = F_d + \xi where m is the mass of the protein, F_d = -\gamma v is the viscous drag and and \xi is the random Brownian force. Note that the Langevin equation is neither deterministic nor symmetric under time-reversal (t \rightarrow -t).

1. Motoring in a “sticky” medium: Purcell’s idea

Using the Stokes formula \gamma = 6\pi r \eta for a globular protein of radius r \sim 10nm moving with an average velocity v \sim 1m/sec (corresponding to 1nm/ns) in the aqueous medium of viscosity \eta \sim 10^{-3} Pas we get an estimate F_d \sim 200pN. Surprisingly, this viscous drag force is about 200 times larger than the elastic force it experiences when stretched by 1nm! Consequently, the Reynold’s number, which is the ratio of the inertial and viscous forces, is at most of the order O(10^{-2}). The Reynold’s number will be of the order of 10^{-2} if you ever try (not recommended) to swim in honey!
Now let us supply the tracks and fuel molecules to the motor protein in the same medium. The Langevin equation will now include additional terms $F_{\text{ext}}$ which represents the net force arising from the interaction of the motor protein with its track and the ligand; the ligand could be the fuel molecule or the molecules produced by its “burning” (e.g., ATP or ADP). Besides, since the dynamics of motor proteins is expected to be dominated by hydrodynamics at low Reynolds’s number $Re$, one generally drops the inertial term. In this “overdamped” regime

$$\gamma v = F_{\text{ext}} + \xi$$  \hspace{1cm} (1)

2. Motoring under random impacts from surroundings: Brownian force

The energy released by the hydrolysis of a single ATP molecule is about $10^{-21}J$. Interestingly, the mean thermal energy $k_B T$ associated with a molecule at a temperature of the order of $T \sim 100K$, is also $k_B T \sim 10^{-21}J$. Moreover, equating this thermal energy with the work done by the thermal force $F_t$ in causing a displacement of $1nm$ we get $F_t \sim 1pN$. This is comparable to the elastic force experienced by a typical motor protein when stretched by $1nm$. Thus, a motor protein that gets bombarded from all sides by random thermal forces is similar to a tiny creature is a very strong hurricane! Therefore, in contrast to the deterministic dynamics of the macroscopic machines, the dynamics of molecular motors is stochastic (i.e., probabilistic).

Already in the first half of the twentieth century D’Arcy Thompson, father of modern bio-mechanics, realized the importance of viscous drag and Brownian forces at the cellular (and subcellular) level. He pointed out that in this microscopic world “gravitation is forgotten, and the viscosity of the liquid,..., the molecular shocks of the Brownian movement, ..., the electric charges of the ionized medium, make up the physical environment ... predominant factors are no longer those of our scale”.

IV. ENERGY TRANSDUCTION BY MOLECULAR MACHINES: FROM CARNOT TO FEYNMAN

Molecular motors generate force by transducing energy. Interestingly, one of the two mechanisms of energy transduction that I describe here, does not have any analogous macroscopic counterpart. I explain in considerable detail why thermodynamic formalisms, which have been successfully utilized to calculate the common performance characteristics of macroscopic machines, are inadequate for natural nano-machines.

Molecular motors are made of soft matter whereas macroscopic motors are normally made of hard matter to withstand wear and tear. Nature seems to exploit the high deformability of molecular motors for its biological function. But, this difference is minor compared to the others and will not be elaborated further here.

A. Molecular machines are run by isothermal engines

This is in sharp contrast to the macroscopic thermal engines which require at least two thermal reservoirs at different temperatures and which convert part of the input heat energy into mechanical work.

Why can’t a molecular machine work as a heat engine? In order to examine the viability of a intracellular heat engine, let us create a temperature gradient on a length scale $\ell \sim 10nm$. An elementary analysis of heat diffusion equation is adequate to show that a temperature gradient on a length scale $\ell$ relaxes within a time interval $\tau_{\text{temp}} \sim c_h \ell^2 / \kappa$ where $c_h$ is the specific heat per unit volume and $\kappa$ is the thermal conductivity. Using the typical characteristic values of $c_h$ and $\kappa$ for water, one finds $[81]$ $\tau_{\text{temp}} \sim 10^{-6} - 10^{-8}s$. Thus, temperature gradients cannot be maintained for the entire duration of even one single cycle of a cyclic molecular machine which is, typically, few orders of magnitude longer than $\tau_{\text{temp}}$. In other words, for all practical purposes, natural nano-machines operate isothermally. Because of the condition $T = \text{constant}$, the molecular machines operate as free energy transducers.

B. Inadequacy of equilibrium thermodynamics

A majority of the molecular motors are chemomechanical machines for which input and output are chemical energy and mechanical work, respectively. Recall that for Carnot’s thermo-mechanical machine, the thermodynamic efficiency is $\eta_{q-th} = 1 - (T_L/T_H)$ where $T_H$ and $T_L$ are the temperatures of the two reservoirs at high and low temperatures, respectively. Similarly, for an isothermal chemo-mechanical engine, the thermal reservoirs would be replaced by chemical reservoirs at fixed chemical potentials $\mu_H$ and $\mu_L$ ($\mu_H > \mu_L$). Consequently, the heat flow of the thermal engine would be replaced by particle flow in the chemical engine. Just as the difference of heat input and output in the heat engine is converted to mechanical work, the difference $\mu_H - \mu_L$ would be converted into output work in the chemical engine. Obviously, the corresponding thermodynamic efficiency would be $[82]$ $\eta_{q-ch} = 1 - (\mu_L/\mu_H)$. Note that, because of the quasi-static nature of each step in the equilibrium thermodynamic theory of these cyclic engines, each cycle takes infinite time. Naturally, the power output $P_{out} = 0$ for both the thermal and chemical Carnot engines.

Can we apply the theory of such isothermal Carnot engines to a chemomechanical molecular machine by identifying, for example, $\mu_H$ and $\mu_L$ ($\mu_H > \mu_L$) as the chem-
ical potentials of ATP and ADP, respectively? The answer is: NO. The cycle time of the real molecular machines is finite and their power output is non-zero. Moreover, motors are examples of open systems which continue to run in repetitive cycles as long as energy is pumped in. Such a system cannot be in thermodynamics equilibrium.

C. Inadequacy of endo-reversible thermodynamics

For macroscopic engines with finite cycle time, the characteristics of performance are often reliably calculated within the theoretical framework of endo-reversible thermodynamics \[84\]. In this case, the ratio of the rates of output and input energies defines the efficiency of transduction. The rate of output work is called power output of the engine.

The formalism of endo-reversible thermodynamics for heat engines is based on the assumption that the working substance of the engine is coupled to the two thermal reservoirs by heat conductors of finite thermal conductivity (non-zero thermal resistance). Entire dissipation is assumed to take place in the irreversible process of heat conduction through these thermal resistors whereas all the processes in the working material of the engine are assumed to be fully reversible (i.e., no entropy is generated internally). In this scenario, the corresponding thermal conductances in the Carnot engine are infinite.

Efficiency at maximum power output \(\eta(\mathcal{P}_{\text{max}})\), rather than maximum efficiency itself, is the most appropriate quantitative measure of the performance of such engines. The upper-bound of \(\eta(\mathcal{P}_{\text{max}})\) is given by the Curzon-Ahlborn expression \[84\] \(\eta_{\text{CA}}(\mathcal{P}_{\text{max}}) = 1 - (T_L/T_H)^{1/2}\). Similarly, within the framework of the endoreversible thermodynamics, a phenomenological theory for chemical engines can also be formulated if the cycle time is finite \[84\]. Can’t we use this approach for molecular machines which are also chemical engines with finite cycle times?

Recall that endo-reversible thermodynamics is based on the assumption that dissipation takes place only in the process of transfer of matter between reservoirs and the working substance whereas processes that the working substance goes through in each cycle are perfectly reversible (and, therefore, non-dissipative). This assumption is valid if the relaxations in the working substance of the engine are very rapid compared to the processes involving the coupling of the working substance with the reservoirs. The validity of this assumption requires clear separation of time scales of relaxation in the working substance and in the working substance-reservoir coupling. But, such separation of time scales does not hold in molecular machines which consist of macromolecules.

D. Domain of non-equilibrium statistical mechanics

In general, molecular motors operate far beyond the linear response regime and, therefore, the formalism of non-equilibrium thermodynamics \[87\] for coupled mechano-chemical processes is not applicable. Moreover, thermodynamic formalisms developed for macroscopically large systems do not account for the spontaneous fluctuations. On the other hand, because of the small size of a molecular motor and because of the low concentrations of the molecules involved in its operation, fluctuations of positions, conformations as well as the cycle times are intrinsic features of their kinetics. Therefore, one has to use the more sophisticated toolbox of stochastic processes and non-equilibrium statistical mechanics for theoretical treatment of molecular machines. Interestingly, as we argue below, noise need not be a nuisance for a motor; instead, a motor can move forward by gainfully exploiting this noise!

E. Defining efficiency: from Carnot to Stokes

The performance of macroscopic motors are characterized by a combination of its efficiency, power output, maximum force or torque that it can generate. Just like the performance of their macroscopic counterparts with finite cycle time, that of molecular motors \[88\] have also been characterized in terms of efficiency at maximum power, rather than maximum efficiency. However, the efficiency of molecular motors can be defined in several different ways \[89\].

The efficiency of a motor, with finite cycle time, is generally defined by

\[
\eta = \frac{\mathcal{P}_{\text{out}}}{\mathcal{P}_{\text{in}}}
\]

(2)

where \(\mathcal{P}_{\text{in}}\) and \(\mathcal{P}_{\text{out}}\) are the input and output powers, respectively. The usual definition of thermodynamic efficiency \(\eta_{\text{th}}\) is based on the assumption that, like its macroscopic counterpart, a molecular motor has an output power \[90\]

\[
\mathcal{P}_{\text{out}} = -F_{\text{ext}}V.
\]

(3)

where \(F_{\text{ext}}\) is the externally applied opposing (load) force. Although this definition is unambiguous, it is unsatisfactory for practical use in characterizing the performance of molecular motors. As explained earlier, a molecular motor has to work against the omnipresent viscous drag in the intracellular medium even when no other force opposes its movement (i.e., even if \(F_{\text{ext}} = 0\)).

A generalized efficiency \(\eta_{G}\) is also represented by the same expression \[2\] where, instead of \(3\), the output power is assumed to be \[91\]

\[
\mathcal{P}_{\text{out}} = F_{\text{ext}}V + \gamma V^2.
\]

(4)

This definition treats the load force and viscous drag on equal footing. In contrast, the “Stokes efficiency” \(\eta_{S}\) for a
molecular motor driven by a chemical reaction is defined as

$$\eta_S = \frac{\gamma V^2}{\langle r \rangle + F_{\text{ext}} V}$$

where $\langle r \rangle$ is the average rate of the chemical reaction and $\Delta G$ is the chemical free energy consumed in each reaction cycle. This efficiency is named after Stokes because the viscous drag is calculated from Stokes law.

As we show in the next subsection, the directional movement of some motors arises from the rectification of random thermal noise. For such motors, an alternative measure of performance is the rectification efficiency $\eta_R$. Thus, different definitions of the efficiency of a molecular motor may arise from different interpretations of their tasks or may characterize distinct aspects of their operation. Nevertheless, for any definition of efficiency, it is essential to ensure that its allowed values lie between 0 and 1.

**F. Noisy power stroke and Brownian ratchet**

If the input energy directly causes a conformational change of the protein machinery which manifests itself as a mechanical stroke of the motor, the operation of the motor is said to be driven by a “power stroke” mechanism. This is also the mechanism used by all man made macroscopic machines. However, in case of molecular motors, the power stroke is always “noisy” because of the Brownian forces acting on it.

Let us contrast this with the following alternative scenario: suppose, the machine exhibits both “forward” and “backward” movements because of spontaneous thermal fluctuations. If now energy input is utilized to prevent “backward” movements, but allow the “forward” movements, the system will exhibit directed, albeit noisy, movement in the “forward” direction. Note that the forward movements in this case are caused directly by the spontaneous thermal fluctuations, the input energy rectifies the “backward” movements. This alternative scenario is called the Brownian ratchet mechanism [93]. The concept of Brownian ratchet [93] was popularized by Richard Feynman with his ratchet-and-pawl device [97]. However, in the context of molecular motors, the Brownian ratchet mechanism was proposed by several groups in the 1990s [94, 95].

Thus, in principle, there are two idealized scenarios for a transition from a conformation A to a conformation B—one is by a pure power stroke and the other by a purely Brownian ratchet mechanism [99]. However, for a real molecular motor, it is difficult to unambiguously distinguish between a power stroke and a Brownian ratchet [101]. The actual mechanism may be a combination of the two idealized extremes.

- **Are Brownian motors Maxwell’s demon?**

Brownian ratchet mechanism transduces random thermal energy of the reservoir into mechanical work. Does it imply that these motors are perpetual motors of the second kind that violate the second law of thermodynamics? In that case, does it have any similarity with the Maxwell’s demon [101]? A short answer is: No, a Brownian ratchet is not a Maxwell’s demon because it is an open system far from the state of equilibrium whereas the second law of thermodynamics is strictly valid only for systems in stable thermodynamic equilibrium.

**G. Physical realizations of Brownian ratchet in molecular motors**

Is there any real biomolecular motor which can be regarded as a true physical realization of Brownian ratchets? The answer is an emphatic “yes” and we list a few typical examples here.

KIF1A, a single-headed kinesin, is an example of cytoskeletal motor whose operational mechanism can be interpreted as a Brownian ratchet [102, 104]. The original acto-myosin crossbridge model suggested by Huxley [105] can be interpreted as Brownian ratchet [106] although Huxley himself did not use this terminology. Brownian ratchet can also account for the force generation by a polymerizing cytoskeletal filament [107].

The crossing of a membrane during the export/import of a protein of length $L$ (amino acid monomers) takes place at a faster rate by the Brownian ratchet mechanism compared to that by pure translational diffusion [108]. It has been claimed that the translocation of a mRNA molecule across the nuclear membrane of an eukaryotic cell takes place also by a similar Brownian ratchet mechanism through the nuclear pore complex [109]. The elongation of a mRNA during transcription by a RNA polymerase can also be a physical realization of the Brownian ratchet mechanism [110, 111]. Translocation, one of the crucial steps of the mechano-chemical cycle of a ribosome is a physical realization of Brownian ratchet [65].

**V. MATHEMATICAL DESCRIPTION OF MECHANO-CHEMICAL KINETICS: CONTINUOUS LANDSCAPES VS. DISCRETE NETWORKS**

We combine the fundamental principles of (stochastic) nano-mechanics and (stochastic) chemical kinetics to formulate the general theoretical framework for a quantitative description of the mechano-chemistry or chemomechanics of molecular motors. We mention a few alternative formalisms.
A. Motor kinetics as wandering in a
time-independent mechano-chemical free-energy
landscape

This approach is useful for an intuitive physical
explanation of the coupled mechano-chemical kinetics of
molecular motors. At least one of the independent co-
dinate axes of this landscape represents the position of
the motor in real space (i.e., its “mechanical coordinate”) while at least another represents its chemical state (i.e.,
“chemical coordinate”). Therefore, the minimum dimen-
sion of this “land” is 2 and the free energy can be re-
presented by the height at each point on the “land”. Both
the position and chemical state variables are assumed
to be continuous. The profiles of the free energy along
both the position and chemical coordinates, obtained by
taking appropriate cross sections of the free energy land-
scape, are periodic with the same periodicity in both the
directions. But, the profile is tilted forward along the
chemical direction so that the bottom of the minima are
located deeper in the forward direction along the “chemi-
cal” coordinate; this tilt accounts for the lowering of free
energy caused, for example, by ATP hydrolysis.

B. Motor kinetics as wandering in the
time-dependent real-space potential landscape

Consider those special situations where the chemical
states of the motor are long lived and change in fast dis-
crete jumps so that the position of the motor can con-
tinue to change without alteration in its chemical state,
except during chemical transitions when position remains
frozen. Thus, no mixed mechano-chemical transition is
allowed in this scenario. In such situations, we can as-
sume that the potential landscape in real space remains
unchanged for a while, during which the wanderings of
the motor in this landscape (i.e., the positional dynamics
of the potential) is governed by the “frozen” spatial profile
of the potential. The profile of the potential switches se-
quentially from one form to another and the sequence of
profiles is repeated in each cycle although the switch-
ing times are random. Different profiles correspond to
different motor-track interactions which is dependent on
the nature of the ligand (if any) bound to the motor.

In this formulation we assume that the allowed po-
sitions of the motor on its track form a continuum x. The
discrete index \( \mu = 1, 2, ..., M \) denote the M dis-

tinct spatial profiles of the potential \( V_\mu(x) \) that are pos-
tulated apriori. At any instant of time \( t \), the state of the
motor is given by \((x, \mu)\). In the overdamped
regime, the time-evolution of the position \( x(t) \) of the
motor obeys the Langevin equation (1) with \[ \boxed{112} \]
\[ F_{ext} = -dV_\mu(x)/dx + F_{load}. \] The time-dependence of the profile
of the potential \( V_\mu(x) \) is governed by the master equation
\[ \frac{\partial P_\mu(x,t)}{\partial t} = \sum_{\mu'} P_{\mu'}(x,t)W_{\mu'\rightarrow\mu}(x) - \sum_{\mu'} P_{\mu}(x,t)W_{\mu\rightarrow\mu'}(x) \]

(6)

where \( P_{\mu}(x,t) \) is the probability that the motor protein
“sees” the landscape \( V_\mu(x) \) at time \( t \) and \( W_{\mu\rightarrow\mu'}(x) \) is the transition probability per unit time for a transition from
the chemical state \( \mu \) to the chemical state \( \mu' \) at position
\( x \).

Instead of the Langevin equation, an equivalent
Fokker-Planck equation can be used to describe the wan-
dering of the motor protein in the potential energy land-
scape \( V_\mu(x) \). The advantage of this alternative formu-
lation is that the Fokker-Planck equation can be com-
bined naturally with the master equation that describes
the time-evolution of the potential energy landscape. In
this hybrid formulation, \( P_{\mu}(x,t) \) represents the proba-
bility that, at time \( t \), the motor is located at \( x \), while “see-
ing” the potential energy landscape \( V_\mu(x) \). The equation
governing the time dependence of \( P_\mu(x,t) \)
\[ \frac{\partial P_\mu(x,t)}{\partial t} = \frac{1}{\eta} \frac{\partial}{\partial x} \left\{ \left[V_\mu'(x) - F \right] P_\mu(x,t) \right\} \]
\[ + \left( \frac{k_B T}{\eta} \right) \frac{\partial^2 P_\mu(x,t)}{\partial x^2} 
+ \sum_{\mu'} P_{\mu'}(x,t)W_{\mu'\rightarrow\mu}(x) 
- \sum_{\mu'} P_{\mu}(x,t)W_{\mu\rightarrow\mu'}(x) \]

(7)

where \( \eta \) is a phenomenological coefficient that captures
the effect of viscous drag. Note that there is no term
in this equation which would correspond to a mixed
mechano-chemical transition.

C. Motor kinetics as a jump process in a fully
discrete mechano-chemical network

In this formulation, both the positions and “internal”
or “chemical”) states of the motors are assumed to be
discrete. Let \( P_\mu(i,t) \) be the probability of finding
the motor at the discrete position labelled by \( i \) and in the
“chemical” state \( \mu \) at time \( t \). Then, the master equation
for \( P_\mu(i,t) \) is given by

\[ \frac{\partial P_\mu(i,t)}{\partial t} = \sum_{j \neq i} \left[ P_{\mu}(j,t)k_{\mu}(j \rightarrow i) - \sum_{j \neq i} P_\mu(i,t)k_{\mu}(i \rightarrow j) \right] 
+ \sum_{\mu'} P_{\mu'}(i,t)W_{\mu'\rightarrow\mu}(i) 
- \sum_{\mu'} \sum_{j \neq i} P_{\mu'}(j,t)W_{\mu'\rightarrow\mu}(j \rightarrow i) 
- \sum_{j \neq i} P_\mu(i,t)\omega_{\mu'\rightarrow\mu}(i \rightarrow j) \]

(8)
where the terms enclosed by the three different brackets \([\cdot]\) correspond to the purely mechanical, purely chemical and mechano-chemical transitions, respectively.

As a concrete example, which will be used also for on several other occasions later in this review, consider a 2-state motor that, at any site \(j\), can exist in one of the only two possible chemical states labelled by the symbols \(1_j\) and \(2_j\). We assume the mechano-chemical cycle of this motor to be

\[
1_j \xrightarrow{\omega_1} 2_j \xrightarrow{\omega_2} 1_{j+1}
\]

where the rates of the allowed transitions are shown explicitly above or below the corresponding arrow. Note that the transition \(1_j \rightarrow 2_j\) is purely chemical whereas the transition \(2_j \rightarrow 1_{j+1}\) is a mixed mechano-chemical transition. The corresponding master equations are given by

\[
\frac{dP_1(i)}{dt} = \omega_2 P_2(i - 1) + \omega_1 P_2(i) - \omega_1 P_1(i)
\]

\[
\frac{dP_2(i)}{dt} = \omega_1 P_1(i) - \omega_1 P_2(i) - \omega_2 P_2(i)
\]

Suppose a load force \(F_{\text{ext}}\) opposes the meacho-chemical transition \(2_j \rightarrow 1_{j+1}\). The effect of the load force can be incorporated by assuming the load-dependence of the corresponding rate constant to be of the form \(\omega_2[F_{\text{ext}}] = \omega_2(0) e^{F_{\text{ext}}/(k_B T)}\) where \(\omega_2(0)\) is the value of the rate constant in the absence of any external force. We’ll see some implications of these equations in several specific contexts later in this article.

### D. Balance conditions for mechano-chemical kinetics: cycles, and flux

On a discrete mechano-chemical state space, each state is denoted by a vertex and the direct transition from one state (denoted by, say, the vertex \(i\)) to another (denoted by, say, the vertex \(j\)) is represented by a directed edge \(|ij|\). The opposite transition from \(j\) to \(i\) is denoted by the directed edge \(|ji|\). A transition flux can be defined along any edge of this diagram. The forward transition flux \(J_{|ij|}\) from the vertex \(i\) to the vertex \(j\) is given by \(W_{jiP_i}\) while the reverse flux \(J_{|ji|}\) from \(j\) to \(i\) is given by \(W_{ijP_j}\). Therefore, the net transition flux in the direction from the vertex \(i\) to the vertex \(j\) is given by \(J_{ji} = W_{ji}P_i - W_{ij}P_j\).

A cycle in the kinetic diagram consists of at least three vertices. Each cycle \(C_{\mu}\) can be decomposed into two directed cycles (or, dicycles) \([113]\) \(C_{\mu}^d\) where \(d = \pm\) corresponds the clockwise and counter-clockwise cycles. The net cycle flux \(J(C_{\mu})\) in the cycle \(C_{\mu}\), in the CW direction, is given by \(J(C_{\mu}) = J(C_{\mu}^+) - J(C_{\mu}^-)\).

For each arbitrary dicycle \(C_{\mu}^d\), let us define the dicycle ratio

\[
\mathcal{R}(C_{\mu}^d) = \frac{\Pi_{<ij>(\mu)\delta C_{\mu}^d} W_{ji}}{\Pi_{<ij>(\mu)\delta C_{\mu}^d} W_{ij}} = \frac{\Pi_{<ij>(\mu)\delta C_{\mu}^d} W_{ji}}{\Pi_{<ij>(\mu)\delta C_{\mu}^d} W_{ij}}\]

(11)

where the superscript \(\mu, d\) on the product sign denote a product evaluated over the directed edges of the cycle.

So, by definition, \(\mathcal{R}(C_{\mu}^-) = 1/\mathcal{R}(C_{\mu}^+).\)

It has been proved rigorously \([114]\) that, for detailed balance to hold, the necessary and sufficient condition to be satisfied by the transition probabilities is

\[
\mathcal{R}(C_{\mu}^d) = \frac{1}{\mathcal{R}(C_{\mu}^d)}\]

(12)

For a non-equilibrium steady state (NESS), one can define \([112]\) the dicycle frequency \(\Omega^\ast(C_{\mu}^d)\) which is the number of dicycles \(C_{\mu}^d\) completed per unit time in the NESS of the system. Then,

\[
\Omega^\ast(C_{\mu}^+)/\Omega^\ast(C_{\mu}^-) = \Pi_{<ij>(\mu)\delta C_{\mu}^d} (W_{ji}/W_{ij}) = \mathcal{R}(C_{\mu}^d)\]

(13)

Clearly, \(\mathcal{R}(C_{\mu}^d) \neq 1\), in general, for any NESS.

Detailed balance is believed to be a property of systems in equilibrium whereas the conditions under which molecular machines operate are far from equilibrium. Does it imply that detailed balance breaks down for molecular machines? The correct answer to this subtle question needs a careful analysis \([112, 117]\). If one naively assumes that the entire system returns to its original initial state at the end of each cycle one would get the erroneous result that the detailed balance breaks down. But, strictly speaking, the free energy of the full system gets lowered by \(|\delta G|\) (e.g., because of the hydrolysis of ATP) in each cycle although the cyclic machine itself comes back to the same state. When the latter fact is incorporated correctly in the analysis \([112, 117]\), one finds that detailed balance is not violated by molecular machines. This should not sound surprising: the transition rates “do not know” whether or not the system has been driven out of equilibrium by pumping energy into it.

### VI. SOLVING FORWARD PROBLEM BY STOCHASTIC PROCESS MODELING: FROM MODEL TO DATA

#### A. Average speed and load-velocity relation

For simplicity, let us consider the kinetic scheme shown in fig. \(\Pi(a)\). In terms of the Fourier transform

\[
\hat{P}_\mu(k, t) = \sum_{j = -\infty}^{\infty} P_\mu(x_j, t) e^{-ikx_j}\]

(14)

of \(P_\mu(x_j, t)\), the master equations can be written as a matrix equation

\[
\frac{\partial \hat{P}(k, t)}{\partial t} = W(k)\hat{P}(k, t)
\]

(15)
where $\vec{P}$ is a column vector of $M$ components ($\mu = 1, \ldots, M$) and $\mathbf{W}(k)$ is the transition matrix in the $k$-space (i.e., Fourier space). Summing over the hidden chemical states, we get

$$\bar{P}(k,t) = \sum_{\mu=1}^{M} P_{\mu}(k,t) = \sum_{\mu=1}^{M} \sum_{j=-\infty}^{\infty} P_{\mu}(x_j, t) e^{-ikx_j}. \quad (16)$$

Taking derivatives of both sides of (16) with respect to $q$ we get [118, 119]

$$i \left( \frac{\partial \bar{P}(k,t)}{\partial k} \right)_{k=0} = < x(t) >$$

$$- \left( \frac{\partial^2 \bar{P}}{\partial k^2} \right)_{k=0} + \left( \frac{\partial \bar{P}(k,t)}{\partial k} \right)_{k=0}^2 = < x^2(t) > - < x(t) >^2$$

Evaluating $\bar{P}(k,t)$, in principle, the stationary drift velocity (i.e., asymptotic mean velocity) $V$ and the corresponding diffusion constant $D$ can be obtained from

$$V = \lim_{t \to \infty} i \frac{\partial}{\partial t} \left[ \left( \frac{\partial \bar{P}(k,t)}{\partial k} \right)_{k=0} \right]$$

$$D = \lim_{t \to \infty} \frac{1}{2} \frac{\partial^2}{\partial t^2} \left[ \left( - \frac{\partial^2 \bar{P}(k,t)}{\partial k^2} \right)_{k=0} + \left( \frac{\partial \bar{P}(k,t)}{\partial k} \right)_{k=0}^2 \right]$$

(17)

Even the forms (20) are not convenient for evaluating $V$ and $D$. Most convenient approach is based on the characteristic polynomial $Q(k)$ associated with the matrix $\mathbf{W}(k)$, i.e., $Q(k;\lambda) = det[\lambda \mathbf{I} - \mathbf{W}(k)]$. Therefore, $\lambda_{\min}(k)$ is a root of the polynomial $Q(k;\lambda)$, i.e., solution of the equation

$$Q(k;\lambda) = \sum_{\mu=0}^{M} C_{\mu}(k) |\lambda(k)|^\mu = 0. \quad (21)$$

Hence [118, 119]

$$V = -i \frac{C_0'}{C_1(0)}$$

$$D = \frac{C_0'' - 2iC_1'(0)V - 2C_2(0)V^2}{2C_1(0)}$$

(22)

where $C_{\mu} = [\partial C_{\mu}(k)/\partial k]_{k=0}$. 

FIG. 1: Three examples of different types of networks of discrete mechano-chemical states. The bullets represent the distinct states and the arrows denote the allowed transitions between the two corresponding states. The scheme is (a) is unbranched whereas that in (b) has branched pathways connecting the same pair of states. The mechanical step size is unique in both (a) and (b) whereas steps of more than one size are possible in (c). (Adapted from fig.1 of ref.[120]).
For a postulated kinetic scheme, $W$ is given. Then the expressions (22) are adequate for analytical derivation of $V$ and $D$ for the given model. However, in order to calculate the distributions of the dwell times of the motors, it is more convenient to work with the Fourier-Laplace transform, rather than the Fourier transform, of the probability densities. Therefore, we now derive alternative expressions for $V$ and $D$ in terms of the full Fourier-Laplace transform of the probability density.

Taking Laplace transform of (14) with respect to time

$$\hat{P}_\mu(k, s) = \int_0^\infty P_\mu(k, t)e^{-st},$$

(23)

the matrix form of the master equation reduces to $\hat{P}(k, s) = R(k, s)^{-1}P(0)$ where $R(k, s) = sI - W(k)$ and $P(0)$ is the column vector of initial probabilities. Now the characteristic polynomial is the determinant of the motors, it is more convenient to work with the Fourier-Laplace transform, rather than the Fourier transform, of the probability densities. Therefore, we now derive alternative expressions for $V$ and $D$ in terms of the full Fourier-Laplace transform of the probability density.

$$|R(k, s)| = \sum_{\mu=0}^M C_\mu(k)s^\mu = 0.$$  

(24)

Equation (24) is formally similar to (21). As expected, we get (21)

$$V = -\frac{\omega_0 - 2\omega_1 V - 2\omega_2 V^2}{\omega_1},$$

$$D = \frac{\omega_0 - 2\omega_1 V - 2\omega_2 V^2}{\omega_1}$$

(25)

**Example: an M-step unbranched mechano-chemical cycle**

As an illustrative example, let us consider the unbranched mechano-chemical cycle (3) with $M = 4$, as shown in fig.2. This special value of $M$ is motivated by the typical example of a kinesin motor for which the four essential steps in each cycle are as follows: (i) a substrate-binding step (e.g., binding of an ATP molecule), (ii) a chemical reaction step (e.g., hydrolysis of ATP), (iii) a product-release step (e.g., release of ADP), and (iv) a mechanical step (e.g., power stroke).

![FIG. 2: An unbranched mechano-chemical cycle of the molecular motor with $M = 4$. The horizontal dashed line shows the lattice which represents the track; $j$ and $j + 1$ represent two successive binding sites of the motor. The circles labelled by integers denote different “chemical” states in between $j$ and $j + 1$. (Adapted from fig.7 of ref. [2].)](image)

Suppose, The forward transitions take place at rates $u_j$ whereas the backward transitions occur with the rates $w_j$. The average velocity $V$ of the motor is given by

$$V = \frac{1}{R_M} \left[ 1 - \prod_{j=0}^{M-1} \left( \frac{w_j}{u_j} \right) \right]$$

(26)

where

$$R_M = \sum_{j=0}^{M-1} r_j$$

(27)

with

$$r_j = \frac{1}{u_j} \left[ 1 + \sum_{k=1}^{M-1} \prod_{i=1}^{k} \left( \frac{w_{j+i}}{u_{j+i}} \right) \right]$$

(28)

while $D$ is given by

$$D = \left[ \frac{(VS_M + dU_M)}{R_M^2} - \frac{(M + 2)V}{2} \right]$$

(29)

where

$$S_M = \sum_{j=0}^{M-1} s_j \sum_{k=0}^{M-1} (k + 1)r_{k+j+1}$$

(30)

and

$$U_M = \sum_{j=0}^{M-1} u_j r_j s_j$$

(31)

while,

$$s_j = \frac{1}{u_j} \left( 1 + \sum_{k=1}^{M-1} \prod_{i=1}^{k} \left( \frac{w_{j+i-1}}{u_{j-i}} \right) \right).$$

(32)

For various extensions of this scheme see ref. [5].

In the simpler case shown in (9), where $M = 2$, and the second step is purely irreversible, using the step size $\ell$ explicitly (to make the dimensions of the expressions explicitly clear), we get

$$V = \ell \left[ \frac{\omega_1 \omega_2}{\omega_1 + \omega_{-1} + \omega_2} \right]$$

$$D = \frac{\ell^2}{2} \left[ \frac{(\omega_1 \omega_2) - 2(V/\ell)^2}{\omega_1 + \omega_{-1} + \omega_2} \right]$$

(33)

Note that if, in addition, $\omega_{-1}$ vanishes, i.e., if both the steps are fully irreversible, then $d/V = \omega_1^{-1} + \omega_2^{-1}$, i.e., the average time taken to move forward by one site is the sum of the mean residence time in the two steps of the cycle.

**Effects of branched pathways and inhomogeneities of tracks**

Microtubule-associated proteins and actin-related proteins can give rise to inhomogeneities of the tracks for cytoskeletal motors. The intrinsic sequence inhomogeneity of DNA and RNA strands can nontrivial influences on the motors that use nucleic acid strands as their tracks [122].
B. Jamming on a crowded track: flux-density relation

In the preceding subsection we considered lone motor moving along a filamentous track. Now we consider a track with heavy motor traffic. In principle, unless controlled by some regulatory signals, formation of “traffic jams” on crowded tracks cannot be ruled out. These phenomena are formally similar to systems of sterically interacting self-propelled particles. One of the simplest theoretical models for such systems is the totally asymmetric simple exclusion process (TASEP). In fact, TASEP was originally inspired by traffic of ribosomes on an mRNA track during translation. However, in recent years it has found application also in the context of traffic-like collective movements of ribosomes in more realistic models as well as many other types of molecular motors, e.g., kinesins, growth of fungal hyphae, growth of bacterial flagella, crowding of deoxyribonucleic acid (DNA) polymerase, and growth of fungal hyphae.

C. Information processing machines: fidelity versus power and efficiency

Power and efficiency are most appropriate quantities to characterize the performance of porters, rowers, sliders, etc. But, not all machines fall in these categories. For information processing machines, which we consider in this subsection, fidelity of information transfer is more important than power and efficiency.

The sequence of the monomeric subunits of a polynucleotide or that of a polypeptide is dictated by that of the corresponding template strand. During the polymerization process, after preliminary tentative selection, the selected monomeric subunit is subjected to several levels of checks by the quality control system of the polymerizing machinery. Only after a selected subunit is screened by such a stringent test, it is covalently bonded to the elongating biopolymer.

Discrimination of monomers based merely on the differences of free-energy gains cannot account for the observed high fidelity of these processes. For example, “kinetic proofreading” has been invoked to explain the kinetic processes that contribute to the high level of translational fidelity. Following three features are essential for kinetic proofreading:

(i) formation of an initial enzyme-reactant complex,
(ii) a strongly forward driven step that results in a high-energy intermediate complex, and
(iii) one or more branched pathways along which dissociation of the enzyme-reactant complex, and rejection of the reactant, is possible before the complex gets an opportunity to make the final transition to yield the product.

Let is consider the kinetic scheme shown below

\[ M_j + P_n + S \rightleftharpoons I_1 \rightarrow I_2 \rightarrow M_{j+1} + P_{n+1} \]

where \( M_j \) denotes the polymerase motor located at the discrete position \( j \) on its template, \( P_n \) represents the elongating biopolymer consisting of \( n \) subunits and \( S \) is a single subunit. Note that the scheme shown in is, at least, formally an extension of an intermediate state \( I_2 \) and a branched path from \( I_2 \) have been added. This scheme is one of the simplest possible implementations of kinetic proofreading.

Kinetic proofreading leads to futile cycles in which at least part of the input free energy is dissipated without elongating the nascent polypeptide by an amino acid monomer. The effects of the consequent loose mechanicochemical coupling of the translational machinery on the rate of polypeptide synthesis has been investigated.

Occasionally wrong monomers escape detection in spite of elaborate selection procedure. In case of DNA replication, the polymerase normally detects the error immediately and corrects it before elongating it further. For this purpose, the nascent DNA is transferred to another specific site on the same polymerase where the wrong monomer is cleaved before transferring the nascent DNA back to the site of elongation activity. The interplay of the two contradictory activities of elongation and shortening of a nascent DNA exposes leads to the coupling of their corresponding rates in the overall rate of DNA polymerization by a DdDP.

D. Beyond average: dwell time distribution (DTD)

Two motors with identical average velocities may exhibit widely different types of fluctuations. Suppose the successive mechanical steps are taken by the motor at times \( t_1, t_2, ..., n, t_n, t_{n+1}, ... \). Then, the time of dwell before the \( k \)-th step is defined by \( \tau_k = t_k - t_{k-1} \). In between successive steps, the motor may visit several “chemical” states and each state may be visited more than once. But, the purely chemical transitions would not be visible in a mechanical experimental set up that records only its position. The number of visits to a given state and the duration of stay in a state in a given visit are random quantities.

In order to appreciate the origin of the fluctuations in the dwell times, let us consider the simple \( N \)-step kinetics:

\[ M_1 \rightleftharpoons M_2 \rightleftharpoons M_3 \rightleftharpoons ... \rightleftharpoons M_j \rightleftharpoons ... \rightleftharpoons M_N \]

Suppose \( t_{\mu,\nu} \) is the duration of stay of the motor in the \( \mu \)-th state during its \( \nu \)-th visit to this state. If \( \tau \) is the dwell time, then

\[ \tau = \sum_{\mu=1}^{N} \sum_{\nu=1}^{n_{\mu}} t_{\mu,\nu} \]
where \( n_\mu \) is the number of visits to the \( \mu \)-th state. It is straightforward to check that

\[
< \tau > = \sum_{\mu=1}^{N} < n_\mu > < t_\mu >
\]

(37)

where \( < n_\mu > \) is the average number of visits to the \( \mu \)-th state and \( < t_\mu > \) is the average time of dwell in the \( \mu \)-th state is a single visit to it. More interestingly \[148\],

\[
< \tau^2 > - < \tau >^2 = \sum_{\mu=1}^{N} ( < n_\mu^2 > - < n_\mu >^2 ) < t_\mu >
\]

\[
+ \sum_{\mu=1}^{N} ( < n_\mu^2 > - < n_\mu >^2 ) < t_\mu >^2
\]

\[
+ 2 \sum_{\mu>\nu} ( < n_\mu n_\nu > - < n_\mu > < n_\nu > ) < t_\mu > < t_\nu >
\]

(38)

The first and second terms on the right hand side of (38) capture, respectively, the fluctuations in the lifetimes of the individual states and that in the number of visits to a kinetic state. Note that the number of visits to a particular state depends on the number of visits to the neighboring states on the kinetic diagram; this interstate correlation is captured by the third term on the right hand side of (38).

Several different analytical and numerical techniques have been developed for calculation of the dwell time distribution \[120, 149–151\]. Since the dwell time is essentially a first passage time \[152\], an absorbing boundary method \[150\] has been used.

1. DTD for a motor that never steps backward

As an example, we consider again the simple scheme \[9\]. In this case, the DTD is

\[
f(t) = \left( \frac{\omega_1 \omega_2}{\omega_+ - \omega_-} \right) (e^{-\omega_+ t} - e^{-\omega_- t})
\]

(39)

where

\[
\omega_{\pm} = \frac{\omega_1 + \omega_{-1} + \omega_2}{2} \pm \left[ \frac{(\omega_1 + \omega_{-1} + \omega_2)^2}{4} - \omega_1 \omega_2 \right]^{1/2}
\]

(40)

In the special case \( \omega_{-1} = 0, \omega_+ = \omega_1 \) and \( \omega_- = \omega_2 \) and, hence,

\[
f(t) = \left( \frac{\omega_1 \omega_2}{\omega_2 - \omega_1} \right) (e^{-\omega_1 t} - e^{-\omega_2 t})
\]

(41)

Similar sum of exponentials for DTD have been derived also for machines with more complex mechano-chemical kinetics (see, for example, refs \[121, 146, 153\]).

It is possible to establish on general grounds that, for a motor with \( N \) mechano-chemical kinetic states like \[155\], the DTD is a sum of \( n \) exponentials of the form \[148\]

\[
f(t) = \sum_{j=1}^{N} C_j e^{-\omega_j t}
\]

(42)

where \( N - 1 \) of the \( N \) coefficients \( C_j \) \( (1 \leq j \leq N) \) are independent of each other because of the constraint imposed by the normalization condition for the distribution \( f(t) \). Also note that the prefactors \( C_j \) can be both positive or negative while \( \omega_j > 0 \) for all \( j \).

If we consider an even more special case of the scheme \[9\] where \( \omega_{-1} = 0, \omega_1 = \omega_2 = \omega \), i.e., all the steps are completely irreversible and take place at the same rate \( \omega \), the DTD becomes the Gamma-distribution

\[
f(t) = \omega^N t^{N-1} e^{-\omega t} \Gamma(N)
\]

(43)

where \( \Gamma(N) \) is the gamma function with \( N = 2 \). Interestingly, for the Gamma-distribution, the randomness parameter \[154\] (also called the Fano factor \[155\])

\[
r = \frac{< \tau^2 > - < \tau >^2}{< \tau >}^{1/2}
\]

(44)

is exactly given by \( r = 1/N \). Can the experimentally measured DTD be used to determine the number of states \( N \)? Unfortunately, for real motors, (i) not each step of a cycle is fully irreversible, (ii) the rate constants for different steps are not necessarily identical, (iii) branched pathways are quite common. Consequently, \( 1/r \) may provide just a bound on the rough estimate of \( N \).

Can one use the general form \[42\] of DTD to extract all the rate constants for the kinetic model by fitting it with the experimentally measured DTD? The answer is: NO. First, even if a good estimate of \( N \) is available, the number of parameters that can be extracted by fitting the experimental DTD data to \[42\] is \( 2N - 1 \) \((n \) values of \( \omega_j \) and \( N - 1 \) values of \( C_j \)). On the other hand, the number of possible rate constants may be much higher \[118\]. For example, if transitions from every kinetic state to every other kinetic state is allowed, the total number of rate constants would be \( N(N-1) \). In other words, in general, the kinetic rate constants are underspecified by the DTD. Second, as the expression \[39\] for the DTD of the example \[9\] shows explicitly, the \( \omega \)'s that appear in the exponentials may be combinations of the rate constants for the distinct transitions in the kinetic model. It is practically impossible to extract the individual rate constants from the estimated \( \omega \)'s unless any explicit relation like \[10\] between the estimated \( \omega \)'s and actual rate constants is a priori available.

2. Conditional DTD for motors with both forward and backward stepping

For motors which can step both forward and backward, more relevant information on the kinetics of a motor are
VII. A SUMMARY OF EXPERIMENTAL TECHNIQUES: ENSEMBLE VERSUS SINGLE-MACHINE

The experimental techniques for probing the structure and dynamics of molecular machines can be divided broadly into two groups: (i) ensemble-averaged, (ii) single-molecule.

Mechanical manipulation of a single biomolecule

Mechanical transducers  Field-based transducers

SFM  Micro-needle  EM-field  Flow-field

Electric field  Magnetic field

VIII. SOLVING INVERSE PROBLEM BY PROBABILISTIC REVERSE ENGINEERING: FROM DATA TO MODEL

A discrete kinetic model of a molecular motor can be regarded as a network where each node represents a distinct mechano-chemical state. The directed links denote the allowed transitions. Therefore, such a model is unambiguously specified in terms of the following parameters: (i) the total number $N$ of the nodes, (ii) the $N \times N$ matrix whose elements are the rates of the transitions among these states; a vanishing rate indicates a forbidden direct transition.

In the preceding sections we handled the “forward problem” by starting with a model that is formulated on the basis of a priori hypotheses which are, essentially, educated guess as to the mechano-chemical kinetics of the motor. Standard theoretical treatments of the model yields data on various aspects on the modelled motor; this approach is expressed below schematically.

Consistency between theoretical prediction and experimental data validates the model. However, any inconsis-
tency between the two indicates a need to modify the model.

In most real situations the numerical values of the rate
costants of the kinetic model are not known a priori. In
principle, these can be extracted by analyzing the experi-
mental data in the light of the model. However, not only
the rate constants but also the number of states and the
overall architecture of the mechano-chemical network as
well as the kinetic scheme postulated by the model may
be uncertain. In that case, the experimental data should
be utilized to “select” the most appropriate model from
among the plausible ones. In fact, more than one model,
based on different hypotheses, may appear to be consistent
with the same set of experimental data within a level
of accuracy. The experimental data can be exploited at
least to “rank” the models in the order of their success
in accounting for the same data set.

The “inverse problem” of inferring the model from the
observed experimental data has to be based on the theory
of probability. Such “statistical inference” can be drawn by
following methods developed by statisticians over the last
one century. Inferring the complete network of mechano-
chemical states and kinetic scheme of a molecular machine
from its observed properties is reminiscent of inferring the
operational mechanism of a given functioning macroscopic machine by “reverse
engineering”. This inverse problem, which is the main
aim of this section, is expressed below schematically.

Theoretical model ← Experimental data

It is worth emphasizing that both the directions of in-
vestigations, i.e. forward problem and the inverse
problem are equally important and complementary to
each other.

A. Frequentist versus Bayesian approach

Suppose, \( \vec{m} \) be a column vector whose \( M 
\) components
are the \( M \) parameters of the model, i.e., the
transpose of \( \vec{m} \) is \( \vec{m}^T = (m_1, m_2, ..., m_M) \). Let the data
obtained in \( N \) observations of this model are represented
by the \( N \)-component column vector \( \vec{d} \) whose transpose is
\( \vec{d}^T = (d_1, d_2, ..., d_N) \). Our “inverse problem” is to infer
information on \( \vec{m} \) from the observed \( \vec{d} \). The philosophy
underlying the frequentist approach, i.e., approaches
based on maximum-likelihood (ML) estimation and the
Bayesian approach for extracting these information are
different in spirit, as we explain in the next two sub-
sections.

For simplicity, let us assume that a device has only two
possible distinct states denoted by \( \mathcal{E}_1 \) and \( \mathcal{E}_2 \).

\[ \mathcal{E}_1 \xrightarrow{\bar{k}_f} \mathcal{E}_2 \] (52)

Let us imagine that we are given the actual sequence of
the states, over the time interval \( 0 \leq t \leq T \), generated
by the Markovian kinetics of the device. But, the magni-
itudes of the rate constants \( k_f \) and \( k_r \) are not supplied.

We’ll now formulate a method, based on ML analysis
\( 169 \) to extract the numerical values of the parameters
\( k_f \) and \( k_r \).

Suppose \( t_j^{(1)} \) and \( t_j^{(2)} \) denote the time interval of the \( j \)-
th residence of the device in states \( \mathcal{E}_1 \) and \( \mathcal{E}_2 \), respectively.
Moreover, suppose that the device makes \( N_1 \) and \( N_2 \) vis-
its to the states \( \mathcal{E}_1 \) and \( \mathcal{E}_2 \), respectively, and \( N = N_1 + N_2 \)
is the total number of states in the sequence. Therefore,
total time of dwell in the two states are \( T_1 = \sum_{j=1}^{N_1} t_j^{(1)} \)
and \( T_2 = \sum_{j=1}^{N_2} t_j^{(2)} \) where \( T_1 + T_2 = T \).

Since the dwell times are exponentially distributed for
a Poisson process, the likelihood of any state trajectory
\( S \) is the conditional probability

\[
P(S|k_f, k_r) = \left( \prod_{j=1}^{N_1} k_f e^{-k_f t_j^{(1)}} \right) \left( \prod_{j=1}^{N_2} k_r e^{-k_r t_j^{(2)}} \right) = \left( k_f^{N_1} e^{-k_f T_1} \right) \left( k_r^{N_2} e^{-k_r T_2} \right) \] (53)

1. Maximum-likelihood estimate

ML approach \( 170 \) is based on finding the estimates
of the set of model parameters that corresponds to the
maximum of the likelihood \( P(\vec{d}|\vec{m}) \) for a fixed set of data
\( \vec{d} \).

For the kinetic scheme shown in equation (52), the the
ML estimates of \( k_f \) and \( k_r \) are obtained by using (53)
in \( d[lnP(S|k_f, k_r)]/dk_f = 0 = d[lnP(S|k_f, k_r)]/dk_r \). It
is straightforward to see \( 169 \) that these estimates are
\( k_f = N_1/T_1 \) and \( k_r = N_2/T_2 \).

2. Bayesian estimate

For drawing statistical inference regarding a kinetic
model, the Bayesian approach has gained increasing pop-
ularity in recent years \( 171, 176 \). The areas of research
where this has been applied successfully include various
biological processes in, for example, genetics \( 177, 178 \),
biochemistry \( 179 \), cognitive sciences \( 180 \), ecology \( 181 \), etc.

In the Bayesian method there is no logical distinc-
tion between the model parameters and the experimental
data; in fact, both are regarded as random. The only
distinction between these two types of random variables is
that the data are observed variables whereas the model
parameters are unobserved. The problem is to estimate
the probability distribution of the model parameters from
the distributions of the observed data.
The Bayes theorem states that

\[ P(\tilde{m}|\tilde{d}) = \frac{P(\tilde{d}|\tilde{m})P(\tilde{m})}{P(\tilde{d})} \]  

(54)

where \( P(\tilde{d}) \) can be expressed as

\[ P(\tilde{d}) = \int P(\tilde{d}|\tilde{m})P(\tilde{m})d\tilde{m} \]  

(55)

The likelihood \( P(\tilde{d}|\tilde{m}) \) is the conditional probability for the observed data, given a set of particular values of the model parameters, that is predicted by the kinetic model. However, implementation of this scheme also requires \( P(\tilde{m}) \) as input. In Bayesian terminology \( P(\tilde{m}) \) is called the prior because this probability is assumed apriori by the analyzer before the outcomes of the experiments have been analyzed. In contrast, the left hand side of equation (54) gives the posterior probability, i.e., after analyzing the data.

Thus, an experimenter learns from the Bayesian analysis of the data. Such a learning begins with an input in the form of a prior probability; the choice of the prior can be based on physical intuition, or general arguments based, for example, on symmetries. Prior choice can become simple if some experience have been gained from previous measurements. Often an uniform distribution of the model parameter(s) is assumed over its allowed range if no additional information is available to bias its choice. To summarize, Bayesian analysis needs not just the likelihood \( P(\tilde{d}|\tilde{m}) \) but also the prior \( P(\tilde{m}) \).

For the kinetic scheme shown in equation (52), the Bayes' theorem (54) takes the form

\[ P(k_f, k_r|S) = \frac{P(S|k_f, k_r)P(k_f, k_r)}{P(S)} = \frac{P(S|k_f, k_r)P(k_f, k_r)}{\sum_{k_f', k_r'} P(S|k_f', k_r')P(k_f', k_r')} \]  

(56)

We now assume a uniform prior, i.e., constant for positive \( k_f \) and \( k_r \), but zero otherwise. Then, \( P(k_f, k_r|S) \) is proportional to the likelihood function \( P(S|k_f, k_r) \) (within a normalization factor). Normalizing, we get

\[ P(k_f, k_r|S) = \left[ \frac{T^{N_1+1}}{\Gamma(N_1+1)} k_f^{N_1} e^{-k_f T_1} \right] \left[ \frac{T^{N_2+1}}{\Gamma(N_2+1)} k_r^{N_2} e^{-k_r T_2} \right] \]  

(57)

The mean of \( k_f \) is \( N_1 + 1 / T_1 \) whereas the most-likely estimate is \( N_1 / T_1 \). Similarly, the mean and most probable estimates of \( k_r \) are obtained by replacing the subscripts 1 by subscripts 2. Moreover, the variance of \( k_f \) and \( k_r \) are \( (N_1 + 1) / T_1^2 \) and \( (N_2 + 1) / T_2^2 \), respectively.

3. Hidden Markov Models

The actual sequence of states of the motor, generated by the underlying Markovian kinetics, is not directly visible. For example, a sequence of states that differ “chemically” but not mechanically do not appear as distinct on the recording of the position of the motor in a single motor experiment. This problem is similar to an older problem in cell biology: ion-channel kinetics [182, 183]. Current passes through the channel only when it is in the “open” state. However, if the channel has more than one distinct closed states, the recordings of the current reveals neither the actual closed state in which the channel was nor the transitions between those closed states when no current was recorded.

Hidden Markov Model (HMM) [184–187] has been applied to analyze FRET trajectories [188, 189], stepping recordings of molecular motors [190, 191], and actomyosin contractile system [192] to extract kinetic information.

For a pedagogical presentation of the main ideas behind HMM, we start with the kinetic scheme shown in [163] and a simple, albeit unrealistic, situation and then by gradually adding more and more realistic features, explain the main concepts in a transparent manner [169]. First, suppose that the actual sequence of states (trajectory) itself is visible; this case can be analyzed either by the ML-analysis of by Bayesian approach both of which we have presented above. We now relax the strong assumption about the trajectory and proceed as below.

- If state trajectory is hidden and visible trajectory is noise-free

The sequence of states of the device is, as before, generated by a Markov process which is hidden. Suppose the device emits photons from time to time that are detected by appropriate photo-detectors. For simplicity, we assume just two detection channels labelled by 1 and 2. For the time being, we also assume a perfect one-to-one correspondence between the state of the light emitting device and the channel that detects the photon. If the channel 1 (2) clicks then the light emitting device was in the state \( E_1 \) (\( E_2 \)) at the time of emission. The interval \( \Delta t_j = t_{j+1} - t_j \) between the arrival of the \( j \)-th and \( j + 1 \)-th photons \((1 \leq j \leq N)\) is random.

Thus, from the photo-detectors we get a visible sequence of the channel index (a sequence made of a binary alphabet) which we call “noiseless photon trajectory” [169]. The sequence of states in the noiseless photon trajectory is also another Markov chain that is conventionally referred to as the “random telegraph process”. Note that the photon detected by channel 1 (or, channel 2) can take place at any instant during the dwell of the device in state 1 (or, state 2). Therefore, the sequence of states in the noiseless photon trajectory does not reveal the actual instants of transition from one state of the device to another.

Since the noiseless photon trajectory corresponds to a random telegraph process, the transition probabilities for
this process are

\[
P(\mathcal{E}_1|\mathcal{E}_1;k_f,k_r,\Delta t_j) = \frac{k_r}{k_f + k_r} + \frac{k_f}{k_f + k_r}e^{-(k_f + k_r)\Delta t_j}
\]

\[
P(\mathcal{E}_1|\mathcal{E}_2;k_f,k_r,\Delta t_j) = \frac{k_r}{k_f + k_r}[1 - e^{-(k_f + k_r)\Delta t_j}]
\]

\[
P(\mathcal{E}_2|\mathcal{E}_1;k_f,k_r,\Delta t_j) = \frac{k_f}{k_f + k_r}[1 - e^{-(k_f + k_r)\Delta t_j}]
\]

\[
P(\mathcal{E}_2|\mathcal{E}_2;k_f,k_r,\Delta t_j) = \frac{k_f}{k_f + k_r} + \frac{k_r}{k_f + k_r}e^{-(k_f + k_r)\Delta t_j}
\]

(58)

where \(P(\mathcal{E}_\mu|\mathcal{E}_\nu;k_f,k_r,\Delta t_j)\) is the conditional probability that state of the device is \(\mathcal{E}_\mu\) given that it was in the state \(\mathcal{E}_\nu\) at a time \(\Delta t\) earlier.

The likelihood of the visible data sequence \(\{V\}\) is now given by

\[
P(\{V\}|k_f,k_r) = P(V_i|k_f,k_r)\Pi_{j=1}^{N-1}P(V_{j+1}|V_j;k_f,k_r,\Delta t_j)
\]

(59)

where the first factor on the right hand side is the initial probability (usually assumed to be the equilibrium probability). The transition probabilities on the right hand side of equation (59) are the conditional probabilities given in equation (58). Unlike the previous simpler case, where the state sequence itself was visible, no analytical closed-form solution is possible in this case. Nevertheless, analysis can be carried out numerically.

**If state trajectory is hidden and visible trajectory is noisy**

In the preceding case of a noise-free photon trajectory, we assumed that from the channel index we could get perfect knowledge of the state of the emitting device. More precisely, the conditional probabilities were

\[
P(1|\mathcal{E}_1) = 1
\]

\[
P(1|\mathcal{E}_2) = 0
\]

\[
P(2|\mathcal{E}_1) = 0
\]

\[
P(2|\mathcal{E}_2) = 1
\]

(60)

However, in reality, background noise is unavoidable. Therefore, if a photon is detected by the channel 1, it could have been emitted by the device in its state \(\mathcal{E}_1\) (i.e., it is, indeed, a signal photon) or it could have come from the background (i.e., it is a noise photon). Suppose \(p_s\) is the probability that the detected photon is really a signal that has come from the emitting device. Suppose \(p_{b1}\) is the probability of arrival of a background photon in the channel 1. The probability that a background photon arrives in channel 2 is \(1 - p_{b1}\). Then

\[
E(1|\mathcal{E}_1) = p_s + (1 - p_s)p_{b1}
\]

\[
E(2|\mathcal{E}_1) = 1 - P(1|\mathcal{E}_1) = (1 - p_s)(1 - p_{b1})
\]

\[
E(1|\mathcal{E}_2) = (1 - p_s)p_{b1}
\]

\[
E(2|\mathcal{E}_2) = 1 - P(1|\mathcal{E}_2) = p_s + (1 - p_s)(1 - p_s)
\]

(61)

Thus, in this case, the relation between the states of the hidden and visible states is not one-to-one, but one-to-many. Therefore, given a hidden state of the device, a set of “emission probabilities” determine the probability of each possible observable state; these are listed in equations (51) for the device (52).

**HMM: formulation for a generic model of molecular motor**

On the basis of the simple example of a 2-state system presented above, we conclude that, for data analysis based on a HMM four key ingredients have to be specified:

(i) the alphabet of the “visible” sequence \(\{\mu\}\) \((1 \leq \mu \leq N)\), i.e., \(N\) possible distinct visible states; (ii) the alphabet of the “hidden” Markov sequence \(\{j\}\) \((1 \leq j \leq M)\), i.e., \(M\) allowed distinct hidden states, (iii) the hidden-to-hidden transition probabilities \(W(j \to k)\), and (iv) hidden-to-visible emission probabilities \(E(j \to \mu)\). In addition to the transition probabilities and emission probabilities, which are the parameters of the model, the HMM also needs the initial state of the hidden variable(s) as input parameters.

Visible: \(V_0 \quad V_1 \quad \ldots \quad V_t \quad V_T\)

Hidden: \(H_0 \quad H_1 \quad \ldots \quad H_T\)

Suppose \(P(\{V\}|HMM,\{\lambda\})\) denotes the probability that an HMM with parameters \(\{\lambda\}\) generates a visible sequence \(\{V\}\). Then,

\[
P(\{V\}|HMM,\{\lambda\}) = \sum_{\{H\}} P(\{V\}|\{H\};\{\lambda\})P(\{H\};\{\lambda\})
\]

(62)

where \(P(\{H\};\{\lambda\})\) is the conditional probability that the HMM generates a hidden sequence \(\{H\}\) for the given parameters \(\{\lambda\}\) and \(P(\{V\}|\{H\};\{\lambda\})\) is the conditional probability that, given the hidden sequence \(\{H\}\) (for parameters \(\{\lambda\}\)) the visible sequence \(\{V\}\) would be obtained.

Once \(P(\{V\}|HMM,\{\lambda\})\) is computed, the parameter set \(\{\lambda\}\) are varied to maximize \(P(\{V\}|HMM,\{\lambda\})\) (for the convenience of numerical computation, often \(\ln P(\{V\}|HMM,\{\lambda\})\) is maximized. The total number of possible hidden sequences of length \(T\) is \(T^{MN}\). In order to carry out the summation in equation (62) one has to enumerate all possible hidden sequences and the corresponding probabilities of occurrences. A successful implementation of the HMM requires use of an efficient numerical algorithm; the Viterbi algorithm is one such candidate.

In case of a molecular motor, the “chemical” states are not visible in a single molecule experiment. Moreover, even its mechanical state that is “visible” in the recordings may not be its true position because of (a) measurement noise, and (b) steps missed by the detector. Let is denote the “visible” sequence by the recorded positions \(\{Y\}\) whereas the hidden sequence is the composite mechano-chemical states \(\{X,C\}\) where \(X\) and \(C\) denote
the true position and chemical state, respectively. The transition probabilities are denoted by $W(X_{i-1}, C_{i-1} \rightarrow X_i, C_i)$. One possible choice for the emission probabilities $E$ is

$$
E(X_i \rightarrow Y_i) = \sqrt{\frac{1}{(2\pi\sigma_i^2)}} e^{-(Y_i - X_i)^2 / (2\sigma_i^2)} \quad (63)
$$

In this case,

$$
P(\{Y\}|HMM, \{\lambda\}) = \sum_{\{X,C\}} P(\{Y\}|\{X,C\}; \{\lambda\}) P(\{X,C\}|\{\lambda\}) \quad (64)
$$

where

$$
P(\{Y\}|\{X,C\}; \{\lambda\}) = E(X_0 \rightarrow Y_0)E(X_1 \rightarrow Y_1)...E(X_i \rightarrow Y_i)...E(X_T \rightarrow Y_T) \quad (66)
$$

The usual strategy consists of the following steps: Step I: Initialization of the parameter values for iteration. Step II: Iterative re-estimation of parameters for maximum likelihood: the parameters $\{W(X_{i-1}, C_{i-1} \rightarrow X_i, C_i)\}$, $\{E(X_i \rightarrow Y_i)\}$ and $P_{X_0, C_0}$ are re-estimated iteratively till $P(\{Y\}|HMM, \{\lambda\})$ saturates to a maximum.

Step III: Construction of "idealized" trace: using the final estimation of the model parameters, the position of the motor as a function of time is reconstructed; naturally, this trace is noise-free. Step IV: Extraction of the distributions of step sizes and dwell times: from the idealized trace, the distributions of the steps sizes dwell times are obtained. These distributions can be compared with the corresponding theoretical predictions.

B. Extracting FP-based model from data?

In this section so far we have discussed methods for extracting the master-equation based models that describe the kinetics of motors in terms of discrete jumps on a fully discrete mechano-chemical state space. However, as we summarized in section IV kinetics of molecular motors can be formulated also in terms of FP equations which treat space as a continuous variable. Can one extract the parameters of such FP-based models from the data collected from single-molecule experiments?

One of the key ingredients of the FP-based approach is the profile of the potential $V_\mu(x)$ felt by the motor in the "chemical" state $\mu$. To my knowledge, it has not been possible to extract it from any experimental data. Now, let us define

$$
d\phi(x)/dx = \frac{1}{\sum_\mu P_\mu(x)} \sum_\mu P_\mu(x) [dV_\mu(x)/dx] \quad (67)
$$

to be the profile of the potential averaged over all the chemical states. It can be shown that

$$
\frac{\phi(x)}{k_BT} = Fxk_BT - \ln \left[ \sum_\mu P_\mu(x) \right] - \frac{J}{D} \int_0^x \frac{1}{\sum_\mu P_\mu(y)} dy \quad (68)
$$

Based on this observation, a prescription has been suggested to extract $\phi(x)$ by analyzing the time series of motor positions obtained from single-motor experiments.

IX. OVERLAPPING RESEARCH AREAS

A. Symmetry breaking: directed motility and cell polarity

Energy is a scalar quantity whereas velocity is a vector. How does consumption of energy give rise to a non-zero average velocity of a molecular motor? Moreover, a directed movement that a motor exhibits on the average, requires breaking the forward-backward symmetry on its track. What are the possible cause and effects of this broken symmetry at the molecular level?

As far as the cause of this asymmetry is concerned, the asymmetry of the tracks alone cannot explain the "directed" movement of the motors, because on the same track members of different families of motors can, on the average, move in opposite directions. Obviously, the structural design of the motors and their interactions with the respective tracks also play crucial roles in determining their direction of motion along a track. Furthermore, this broken symmetry at the molecular level, e.g., the “directed” movement of molecular motors, has important effects on various biological phenomena, particularly "vectorial" processes, at the sub-cellular and cellular levels. In general, a cell is not isotropic. Motors are essential in breaking the cellular symmetry. Can we establish a unique set of basic principle underlying the symmetry breaking?
Therefore, the cause and effects of broken symmetry of molecular motors can be examined in the broader context of the fundamental principles of symmetry breaking in physics and biology \[198\] (see also other articles in the special “Perspectives on Symmetry Breaking in Biology” \[197\]). For macroscopic systems in thermodynamic equilibrium, symmetry breaking is explained in terms of the form of the free energy. However, since living cells are far from thermodynamic equilibrium, the theory of symmetry breaking in those systems cannot be based on thermodynamic free energy. Kinetics cannot be ignored in the study of symmetry breaking in living systems.

B. Self-organization and pattern formation: assembling machines and cellular morphogenesis

The interior of a living bacterial cell is far from homogeneous; the intracellular space of eukaryotes are divided into separate compartments. Scaling is one of the interesting properties of many physical quantities that gives rise to some well known universalities. The scaling properties of a cell and the subcellular compartments \[195, 199\] often depend on the machineries which assemble them.

The size, shape, location and number of intracellular compartments as well as modular intracellular machineries are self-organized, rather than self-assembled. Dissipation takes place in “self-organization” and distinguishes it from “self-assembly”; the latter corresponds to the minimum of thermodynamic free energy whereas self-organized system does not attain thermodynamic equilibrium \[195, 200–202\]. Molecular motors and their filamentous tracks play important roles in the intracellular self-organization process \[195, 201\] and even in the cellular morphogenesis which may be regarded as a problem of pattern formation far from equilibrium.

C. Dissipationless computation: polymerases as “tape-copying Turing machines”

The concept of information in the context of biological systems has been discussed at length in the past \[203\]. The operation of molecular machines involved in genetic processes can be analyzed in terms of storage and transmission of information. In fact, a particular class of machines carry out what may be viewed as data transmission whereas that of others may be viewed as digital-to-analog conversion \[204\] . For these obvious reasons, a broad class of molecular machines are interesting also from the perspective of information theory, electronic engineering and computer science.

Computation can be viewed as a transition from one state to another. However, in a conventional digital computer an elementary operation is logically irreversible. To understand the meaning of this term, consider the two summations \(3 + 1 = 4\) and \(2 + 2 = 4\). If the computer retains only the output, i.e., 4 and erases the input numbers (i.e., 3 and 1, or 2 and 2, as the case may be) after summing the two input numbers, then, given only the output (i.e., 4) it is impossible to figure out whether the input were 3, 1 or 2, 2. Note that erasing every bit leads to loss of information which may also be interpreted as an increase of entropy. However, strategies for logically reversible computation have been developed \[205, 206\].

For example, the simplest strategy for logically reversible computation is based on the prescription that neither the initial input nor the data in any intermediate step should be erased; instead, these should be retained in an auxiliary register.

In practice, computation is carried out with a device that is governed strictly by the laws of physics that includes thermodynamics. Since entropy increases in every irreversible physical process, it would be tempting to associate the logical irreversibility of computation with a physical irreversibility of the computational device. Since each bit of a classical digital computer has only two possible states, at first sight, one would expect dissipation of \(k_B T \ln 2\) energy for every bit erased \[207\]. But, the possibility of logically reversible computation raises a fundamental question: is it possible for a physical computing device to carry out physically reversible (and, hence non-dissipative) computation?

Operation of a polymerase (and that of a ribosome) can be regarded as computation. More precisely, such a computing machine can be viewed as a “tape-copying Turing machine” that polymerizes its output tape, instead of merely writing on a pre-synthesized tape \[206\]. Dissipationless operation of these machines is possible only if every step is error-free which, in turn, is achievable in the vanishingly small speed, i.e., reversible limit \[208\].

D. Enzymatic processes: conformational fluctuations, static and dynamic disorder

For a motor that doesn’t step backwards, the position advances in the forward direction one step at a time; however, the time gap between the successive steps, i.e., dwell time, is a random variable. Similarly, in single-molecule enzymology, the population of the product molecules increases by one in each enzymatic cycle, the time gap between the release of the successive product molecules is random. The distribution of this time \[209, 210\] is analogous to that of the dwell times of molecular motors \[120\]. Therefore, the research fields of single-motor mechanics and single-enzyme reactions can enrich each other by exchange of concepts and techniques \[148\].

As a concrete example, consider the chemical reaction

\[
E + S \overset{\omega_1}{\underset{\omega_2}{\iff}} I_1 \overset{\omega_3}{\rightarrow} E + P
\]

(69)
catalyzed by the enzyme \(E\) where \(S\) and \(P\) denote the substrate and product, respectively. The enzyme and
the substrate form, at a rate $\omega_1$, an intermediate complex $I_1$ that can either dissociate into the free enzyme and the substrate at a rate $\omega_{-1}$, or get converted irreversibly into the product and free enzyme at a rate $\omega_2$. In a bulk sample, where a large number of enzymes convert many substrates into products by catalyzing this reaction simultaneously, the measured rate of the reaction is actually an average over the ensemble. This rate was calculated by Michaelis and Menten about 100 years ago and is given by the celebrated Michaelis-Menten equation \[ \frac{V}{[S]} = \frac{V}{K_m} + \frac{[S]}{V} \] 

Formally, the scheme (69) is very similar to the scheme (49) that we presented earlier as a very simple example of the mechano-chemical cycle of a molecular motor. The time taken by the chemical reaction (69) fluctuates from one enzymatic cycle to another. One of the fundamental questions is: what are the conditions under which the average rate would still satisfy the Michaelis-Menten equation \[ \frac{V}{[S]} = \frac{V}{K_m} + \frac{[S]}{V} \] ?

Another topic that overlaps research on molecular motor kinetics and chemical kinetics is *allosterism* \[212\]. A motor protein has separate sites for binding the fuel molecule and the track. How do these two sites communicate? How does the binding of ligand at one site affect the binding affinity of the other? The mechanochemical cycle of a motor can be analyzed \[213\] from the perspective of allosterism which is one of the key mechanisms of cooperativity in protein kinetics \[214\].

E. Applications in biomimetics and nano-technology

Initially, technology was synonymous with macro-technology. The first tools applied by primitive humans were, perhaps, wooden sticks and stone blades. Later, as early civilizations started using levers, pulleys and wheels for erecting enormous structures like pyramids. Until nineteenth century, watch makers were, perhaps, the only people working with small machines. Using magnifying glasses, they worked with machines as small as 0.1 mm. Micro-technology, dealing with machines at the length scale of micrometers, was driven, in the second half of the twentieth century, largely by the computer miniaturization

In 1959, Richard Feynman delivered a talk \[215\] at a meeting of the American Physical Society. In this talk, entitled “There’s Plenty of Room at the Bottom”, Feynman drew attention of the scientific community to the unlimited possibilities of manipulating and controlling things on the scale of nano-meters. This famous talk is now regarded by the majority of physicists as the defining moment of nano-technology \[216\]. In the same talk, in his characteristic style, Feynman noted that "many of the cells are very tiny, but they are very active, they manufacture various substances, they walk around, they wiggle, and they do all kinds of wonderful things all on a very small scale". From the perspective of applied research, the natural molecular machines opened up a new frontier of nanotechnology \[217, 222\]. The miniaturization of components for the fabrication of useful devices, which are essential for modern technology, is currently being pursued by engineers following mostly a top-down (from larger to smaller) approach. On the other hand, an alternative approach, pursued mostly by chemists, is a bottom-up (from smaller to larger) approach.

Unlike man-made machines these are products of Nature’s *evolutionary design* over billions of years. In fact, cell has been compared to an “archeological excavation site” \[223\], the oldest modules of functional devices are the analogs of the most ancient layer of the exposed site of excavation. We can benefit from Nature’s billion year experience in nano-technology. The term *biomimetics* has already become a popular buzzword \[224, 225\]; this field deals with the design of artificial systems utilizing the principles of natural biological systems.

X. CONCEPT OF BIOLOGICAL MACHINES: FROM ARISTOTLE TO ALBERTS

The concept of “living machine” evolved over many centuries. Some of the greatest thinkers in human history made significant contributions to this concept. It started with the man-machine (and, more generally, animal-machine) analogy. Aristotle \[224\] distinguished between the “body” and the “soul” of an organism. However, after more than one and half millenia, an intellectually provocative idea was put forward by Rene Descartes when he argued that animals were “living machines”. This idea took its extreme form in Julien Offray de La Mettrie’s book \[225\] L’homme Machine (‘man a machine’). Leibnitz \[226\] wrote that the body of a living being is a kind of “divine machine or natural automaton” and it “surpasses all artificial automata”, because not each part of a man-made machine is itself a machine. In contrast, he argued, living bodies are “machines in their smallest parts ad infinitum”. Is this statement to be interpreted as Leibnitz’s speculation for the existence of machines within machines in a living organism? The debate over this interpretation continues \[227, 228\].

All the great thinkers from Aristotle to Descartes and Leibnitz compared the whole organism with a machine, the organs being the coordinated parts of that machine. Cell was unknown; even micro-organisms became visible only after the invention of the optical microscope in the seventeenth century. Marcelo Malpighi, father of microscopic anatomy, speculated in the 17th century about the existence molecular machines in living systems. He wrote (as quoted in english by Marco Piccolino \[229\]) that the organized bodies of animals and plants been constructed with “very large number of machines”. He went on to characterize these as “extremely minute parts so shaped and situated, such as to form a marvelous organ”. Unfortunately, the molecular machines were invisible not
only to the naked eye, but even under the optical microscopes available in his time. In fact, individual molecular machines could be “caught in the act” only in the last quarter of the 20th century. We highlight here the progress made during the last three centuries when gradually the analogy with machine got extended to cover all levels of biological organization—from the topmost level of organisms down to cellular and subcellular levels. Not surprisingly, muscles seem to have attracted maximum attention in the context of machinery of life.

Thomas Henry Huxley, dubbed as “Darwin’s bulldog” for his strong support for Darwinian ideas of evolution, delivered his famous lecture, titled “On the Physical Basis of Life,” on 8th November, 1868 (see ref. [9] for the published version). Among the provocative statements and insightful comments in this article, which received lot of hostile criticism at that time, I quote only a few that are directly relevant from the perspective of molecular machines. Huxley had the foresight to see that [9] “speech, gesture, and every other form of human action are, in the long run, resolvable into muscular contraction, and muscular contraction is but a transitory change in the relative positions of the parts of a muscle”—a remarkably insightful comment on contractility and motility because the mechanism of muscle contraction was discovered almost 90 years later, one of the discoverers being his grandson!

David Ferrier, a pioneering neurologist and a younger contemporary of Thomas Huxley, in his lecture delivered at the Middlesex Hospital Medical School, on October 5th, 1870, not only echoed similar ideas but stressed the role of physical energy, rather than any hypothetical vital action, for sustaining life of an organism. A few years later, Robert Henry Thurston, the first president of the ASME (American Society of Mechanical Engineers) and a pioneer of modern engineering education, investigated what he called a “vital machine” (or “prime motor”) from an engineer’s perspective.

In the initial stages, most of the visionaries compared animals with a machine. Although movements of plants received much less attention, results of pioneering systematic studies of these phenomena were reported already in the nineteenth century by Charles Darwin in a classic book which was co-authored by his son. Later, in the preface of his report on the classic investigations on the mechanical response of plants to stimuli, Jagadis Chandra Bose wrote: “From the point of view of its movements a plant may be regarded ...simply as a machine, transforming the energy supplied to it, in ways more or less capable of mechanical explanation”. Interestingly, the first chapter was titled “The plant as a machine”.

Based on the well known fact that all animals exhibit irritability and contractility, Thomas Henry Huxley had already speculated in ref. [9], that “it is more than probable, that when the vegetable world is thoroughly explored, we shall find all plants in possession of the same powers.” In support of this possibility he described a phenomenon that is now known as cytoplasmic streaming. His speculation that “the cause of these currents lie in contraction” was established a century later when cytoplasmic streaming was shown to be caused by an acto-myosin system.

“The story of the living machine” narrated by Herbert William Conn, one of the founding members and the third president of the Society of American Bacteriologists (renamed, in 1960, American Society for Microbiology), is a critical overview of the understanding of these machines at the end of the nineteenth century. Because of his deep understanding of the basic principles of physical sciences as well as evolutionary biology, his narrative on the fundamental principles of living machines remains as contemporary today as it was at the time of its publication. Conn asked whether the operations of individual organisms, i.e., the living machines, could be “reduced to the action of still smaller machines.” Conn argued that one can look upon each constituent cell of an organism also “a little engine with admirably adapted parts.” His summary that a living organism is a “series of machines one within the other” sounds very similar to Leibnitz’s philosophical idea. Conn went even further: “As a whole it is machine, and its parts are separate machines. Each part is further made up of still smaller machines until we reach the realm of the microscope. Here still we find the same story. Even the parts formerly called units, prove to be machines”. He speculated “we may find still further machines within” cells. He ended his summary with the statement “And thus vital activity is reduced to a complex of machines, all acting in harmony with each other to produce together the one result—life.”

Conn not only discussed “the running of the living machine”, but also “the origin of the living machine”. In the latter context, while pointing out the differences in the principles of engineering design of man-made machines and evolutionary principles of nature’s nano-machines, he wrote: “It is something as if steam engine of Watt should be slowly changed by adding piece after piece until there was finally produced the modern quadruple expansion engine, but all this change being made upon the original engine without once stopping its motion.”

Jaques Loeb, a famous embryologist, delivered a series of eight lectures at the Columbia university in 1902 (see ref. [236] for a more complete published version). In the first lecture, Loeb started by saying “In these lectures we shall consider living organisms as chemical machines, consisting essentially of colloidal material, which possess the peculiarities of automatically developing, preserving, and reproducing themselves”. He emphasized the crucial differences between natural and artificial machines by the following statement: “The fact that the machines which can be created by man do not possess the power of automatic development, self-preservation, and reproduction constitutes for the present a fundamental difference between living machines and artificial ma-
chines”. However, just like some of his other visionary predecessors, he also admitted that “nothing contradicts the possibility that the artificial production of living matter may one day be accomplished” 230.

The concept of “living machine” as a “transformer of energy”, from the level of a single cell to the level of a multi-cellular organism as complex as a man, found mention in many lectures and books of the leading biologists in the late nineteenth and early twentieth centuries (see, for example, 237). Research on molecular machines was focussed almost exclusively on the mechanism of muscle contraction during the first half of the 20th century and it was dominated by Archibald Hill and Otto Meyerhof 238, 239 who shared the Nobel prize in Physiology (or medicine) in 1922.

By mid-twentieth century, physical sciences already witnessed spectacular progress and life sciences was just embarking on its golden period. In his Guthrie lecture (also titled “The Physical Basis of Life”), delivered on 21st November, 1947 (see ref. 240 for the full text), J. D. Bernal drew attention of the audience to the structure and dynamics of machines. Ten years later, in a review article, the title of which again had the words “physical basis of life”, Schmitt 241 made even more concrete references to molecular machines covering almost all the types of machines that we have sketched in this article.

The concept of molecular machines 242 was mentioned on many occasions in the late twentieth century by leading molecular biologists who made outstanding contributions in elucidating their mechanisms of operation. The strongest impact was made, however, by the influential paper of Bruce Alberts 243, then the president of the National Academy of Sciences (USA). He wrote that “the entire cell can be viewed as a factory that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein machines” 243.

Why did I start my story with Aristotle? In D’Arcy Thompson’s words 244 “We know that the history of biology harks back to Aristotle by a road that is straight and clear, but that beyond him the road is broken and the lights are dim”. Moreover, a section of the modern enterprise in animal sciences is pursuing important investigations on the efficiency of energy utilization by animals 245 treating an entire animal as a “combustion engine”, a modern and scientifically correct version of the original philosophical idea developed by Aristotle and propagated by his followers.

Why am I closing my story with Alberts? Because of a fortuitous coincidence, Alberts’ vision for training the next generation of biologists for studying natural nano-machines coincided with the explosive beginning of nanotechnology that includes research programs on artificial nano-machines. Alberts’ article 243 was appeared at a time when the statistical physics of Brownian ratchets was getting lot of attention 94. The concept of molecular machine has matured fully into an area of interdisciplinary research in the twenty-first century. A new exciting era of research has just begun!

XI. SUMMARY AND OUTLOOK

So far as the current status of our understanding is concerned, I would say that till the middle of the 20th century we had never seen or manipulated a single molecular machine although machine-like operation of a cell or an entire organism was fairly well established. In the last 60 years this area has seen explosive growth in activity. The structures as well as the mechanisms of many machines no longer appear any more mysterious than those of macroscopic machines.

It is practically impossible to predict new ideas in any field of research; molecular machines are no exception. The reason, as Peter Medawar admitted in his presidential address at the Cambridge meeting of the British Association for the Advancement of Science, is as follows: “to predict an idea is to have an idea, and if we have an idea it can no longer be the subject of prediction”. Therefore, in this section, I do not propose any new idea but merely mention a few systems and phenomena which need new ideas for their studies and understanding.

Let me begin with solving the forward problem with process modeling. Here we need new ideas to make progress in two opposite directions: (a) in-silico modeling of single individual machines in terms of its coordinat- ing parts in an aqueous medium- aquatic nano-robotics; (b) integration of nano-machines and machine-assemblies into a micro-factory, the living cell. So far as the point (a) is concerned, serious efforts have been made by developing coarse-grained computational models 247–249 that can be regarded as the substitutes for full molecular dynamics based models. However, a technical (or algorithmic) breakthrough is needed to achieve the ultimate goal of “seeing” the operation of a machine in its natural aqueous environment by carrying out experiments in-silico.

Next let me explain the aim of developing integrated models. It has been strongly argued at the dawn of this millenium 250 that the machinery of life is modular: The machineries of transcription, translation, replication, chromosome segregation, etc. are all examples of modules which perform specific tasks. The components of some of these modules are parts of a single machine, e.g., all devices participating in translation function on a single platform provided by a ribosome. Signal transduction machinery is an extreme example of the other types of functional modules which are spatially distributed over a significantly large region of intracellular space without need for direct physical contacts among the parts.

The machines that we have considered here are all more or less spatially-confined functional modules. Normally, functional modules are not completely isolated and must communicate and coordinate their function with other modules. For example, DNA replication and chromosome segregation machineries require proper coordi-
nation. To my knowledge, no attempt has been made so far for quantitative stochastic process modeling incorporating more than one functional module in a seamless fashion. Such an enterprise may look like what is now happening in systems biology: to integrate the operations of machineries for different functional modules within a single theoretical framework.

Finally, let me point out some of the standard practices of statistical inference that, to my knowledge, have not been followed so far while reverse-engineering molecular machines. The experimental data for molecular machines have been analyzed by several groups to extract an underlying kinetic model. However, most often the analysis carried out during such reverse engineering is based on a single working hypothesis. It would be desirable to follow Platt’s principle of “strong inference” which is an extension of Chamberlin’s “method of multiple working hypothesis”. By multiple model I do not mean models hierarchically nested such that each one is a special case of the model at the next level. By the term multiple model, I mean truly competing models that may, however, overlap partially. The relative scores of the competing models (and the corresponding underlying hypothesis) would be a true reflection of their merits. For example, in case of a closely related systems in cell biology and systems biology, experimental data have been analyzed recently within the framework of this method. In the case of molecular machines, a method for model selection has been developed; it extracts the best model by optimizing the maximum evidence, rather than maximum likelihood, that treats, for example, the number of discrete states of the system as a variational parameter. This is a step in the right direction. However, I am not aware of any work that ranks alternative models according to relative scores computed on the basis of the principle of strong inference.

The molecular mechanism of muscle myosin and the structure of double-stranded DNA were both discovered in the 1950s. These discoveries set the stage for the research on cytoskeletal motors as well as on machines that make, break and manipulate nucleic acids and proteins. Last 50 years has seen enormous progress. I wanted to tell you about the PIs and their contributions during this glorious period. But, I have run out of space. So, I’ll narrate this fascinating story in detail elsewhere.

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