Natural Nano-Machines

2. Discussion on Methods, Materials and Mechanisms

Debashish Chowdhury

In the first part of this article, we read about Alice's guided tour through the cellular micro-factory. In the second part, I introduce the methods of studying the materials and mechanisms of the molecular machines through dialogues. The three participants in this discussion are Alice, her elder brother Alex and her father Albert. The style of presentation here is adapted from Galileo's *Dialogue Concerning the Two Chief World Systems*. Albert, a professor of biophysics, emphasizes the crucial differences between the mechanisms of the natural nano-machines and those of their macroscopic counterparts. He also points out some practical applications of this interdisciplinary research in biomedical science and nano-technology.

Debashish Chowdhury is a professor of Physics at IIT Kanpur. He is a theoretical physicist and the areas of his research interest are statistical and biological physics. He plays with toy models using mathematical formulae and simulates nature with computers.

Alice: Can you really see an individual molecular machine with the help of some equipment in your lab as clearly as I saw them in my dream?

Albert: Seeing is believing. However, you may be surprised to hear that until late in the 20th century scientists could not see individual molecules although everybody believed in their existence. In fact, in those days, the existence of a molecule could only be inferred indirectly from some circumstantial evidences gathered from experiments on samples which used to be very large compared to single molecules. Search for microscopes which would allow us to see this elusive object seemed like a "holy grail". We got our first glimpse of the macromolecules via X-ray diffraction and then, electron microscopy.

A popularized version of the introductory lecture in an undergraduate course on '*Natural Nano-machines*' taught at IIT, Kanpur.

Part 1. Natural Nano-Machines – Alice in a Micro-Factory, Vol.12, No.1, pp.4-14, 2007.

Keywords
Motor protein, intra-cellular transport, ion pump, ATP synthase.

Optical microscopy uses light waves whereas the wave nature of electrons is utilized in electron microscopy.

Alice: Are the basic underlying principles of electron microscopy different from those of optical microscopy?

Albert: No. The basic principles of these are essentially similar. Optical microscopy uses light waves whereas the wave nature of electrons is utilized in electron microscopy. The main difference is that a higher spatial resolution is achievable by electron microscopy because of the smaller wavelength of the electrons.

Alex: Why were scientists not satisfied even with the structures determined by electron microscopy?

Albert: What one got from those probes were static pictures. Towards the end of the 20th century, a series of novel imaging techniques emerged which revolutionized optical microscopy. With the help of these tools we can now monitor the dynamics of single-molecules [1]. Confocal fluorescence microscopy is, perhaps, the most versatile among these methods. The confocal microscope focusses on the intended plane of a sample and filters out the light from all other planes.

Alex: Are these extraordinary developments in experimental techniques mainly responsible for the recent revolutions in cell biology?

Albert: Yes, indeed. Invention of novel experimental techniques always leads to enormous progress in natural sciences. For example, inventions of the telescope, microscope, X-ray opened up new horizons. In order to understand the mechanisms of molecular machines we need experimental probes with sufficiently high *spatial* as well as *temporal* resolutions that would be adequate to watch the dynamics of these machines. Such high resolutions have been attained only in the last few years.

The confocal microscope focusses on the intended plane of a sample and filters out the light from all other planes.

Alice: What are these new experimental techniques for watching single molecules?

Albert: The recently developed single-molecule tech-

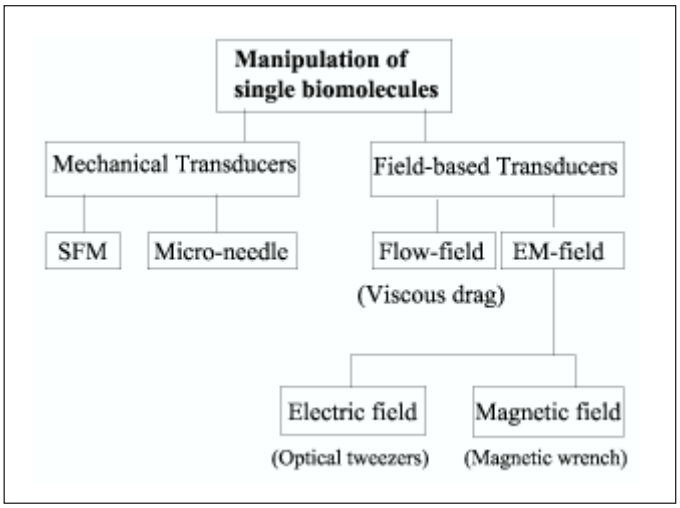

Figure 1. A broad classification of the experimental techniques available for the manipulation of single molecules.

niques can be broadly classified into two groups: (i) methods of imaging, and (ii) methods of manipulation. For example, green (and red) fluorescence microscopy enables us to monitor the dynamics of single motors just as ecologists use "radio collars" to track individual animals. But, for a clear understanding of the mechanism of the molecular machines it is not enough merely to watch them passively; we must also be able to manipulate them (see *Figure* 1). Such manipulations have been possible over the last decade because of the availability of sophisticated techniques like, for example, optical tweezers and atomic force microscopes (AFM).

Alice: Is there any similarity between 'optical tweezer' and the tweezer grandma uses for pulling out grandpa's grey hairs?

Albert: Although the two look very different, their functions are very similar. In optical tweezers, oppositely moving laser beams create an optical trap using the radiation pressure of the laser light; this trap can grab a dielectric object which then can be manipulated just like what your grandma does with her tweezer.

Alex: What is the working principle of AFM?

Albert: The scanning force microscope (SFM) was originally designed to image a surface by scanning it with a sharp tip just like our finger tip that gives a fair picture of the macroscopic irregularities and the overall topography of a surface. In the AFM the tip is replaced by a cantilever that can pull, for example, a motor protein.

Alice: I thought only biologists study whatever goes on inside a cell. I didn't know that physicists like you are also working on these phenomena.

Albert: That, indeed, was true till a couple of decades ago. But, now, this is a frontier area of interdisciplinary research that involves molecular cell biology, biological chemistry, physics as well as engineering, especially nano-technology [2].

Alice: What is nano-technology?

Albert: Technologies which suitably utilize structures and processes that occur at the nanometer scale are collectively referred to as 'nanotechnology'. For developing nanotechnology it is not enough just to get a fundamental understanding of structures and dynamics of materials at nanometer scale but one requires techniques to manipulate matter on such short scales so that novel applications can be innovated.

Alex: Is nano-technology a very recent development?

Albert: Although quest for ever-smaller size of devices began long ago, Feynman's famous talk of 1959 [3] is accepted by the majority of physicists as the defining moment of nano-technology. Feynman first attracted the attention of the physics community to the unlimited possibilities of manipulating and controlling things on the scale of nanometers. He indicated the potential advantages of nano-technology. He also speculated on the possible methods. Nano-technology is the latest trend in the 21st century.

Feynman's famous talk of 1959 [3] is accepted by the majority of physicists as the defining moment of nano-technology.

Alex: What is the main goal of physicists working in this area of research?

Albert: Physicists use several different methods of investigation, namely, experimental, theoretical and computational techniques, for discovering the common fundamental physical principles that govern the generic features of the structure and dynamics of biological nanomachines. They also investigate the corresponding underlying mechanisms of operations of specific machines.

Alex: I understand the term 'experimental method' and you have already given some examples. But, I don't understand the term 'theoretical method'.

Albert: By 'theoretical methods' we mean analytical (i.e., mathematical) treatment of theoretical models. Since such an analysis can be accomplished exactly only in rare cases, one has to make sensible approximations so as to get quantitative results as accurately as possible.

Alice: My biology teacher told us that the fruit fly *Drosophila* is a 'model organism'. Is there any relation between 'model organism' and 'theoretical model'?

Albert: No. In biology, often the simplest among a family of organisms is called a model system for the purpose of experimental investigations. For example, the fruit fly *Drosophila* and the worm *C. elegans* are often called model organisms because of their simpler features among all multi-cellular organisms. However, a theoretical model is an abstract representation of the real system. Biologists often develop *qualitative* models to explain empirical observations. In contrast, physicists work mostly with *quantitative* models, formulated in terms of mathematical symbols, not only to interpret experimental data but also to make new predictions that can be tested through experiments.

The fruit fly *Drosophila* and the worm *C. elegans* are often called model organisms because of their simpler features among all multi-cellular organisms.

Alex: Wouldn't it be wise to develop an all encompass-

Every theoretical model is intended to address a set of questions. The modeler must choose a *level of description* appropriate for this purpose keeping in mind the phenomena that are being investigated.

ing single theoretical model that can explain all the intracellular phenomena?

Albert: Every theoretical model is intended to address a set of questions. The modeler must choose a *level of description* appropriate for this purpose keeping in mind the phenomena that are being investigated. Otherwise, the model may have either too much redundant details or it may be too coarse to provide any useful insight. For example, a molecular model of a hair dryer will have too much redundant information. Similarly, a continuum model of liquid water will be too coarse to study the dynamics of individual molecules of water. Since physicists most often focus only on generic features of the various classes of machines, rather than specific features of individual members of these classes, they normally develop minimal models which may be regarded as *mesoscopic*, rather than molecular, i.e., their status is somewhere between those of the macroscopic and molecular models.

Alex: So far as the computational methods are concerned, are these identical to those used, for example, in bioinformatics?

Albert: Computational biology has two distinct branches:

(i) **Knowledge discovery** (or, *data mining*) which extracts hidden patterns or laws from huge quantities of experimental data, forming hypotheses. Knowledge discovery is used extensively in bioinformatics.

(ii) **Simulation-based analysis**, which tests hypotheses with computer simulations, provides predictions that, at least in principle, can be tested by *in vitro* and/or *in vivo* experiments in the laboratory. The starting point of a computer simulation is to develop an *algorithm*, which can be implemented numerically, for calculating the desired quantities. The sequence of instructions to a computer specifying the numerical procedure of that

algorithm constitutes a computer *program*.

Alice: Are computer simulations different from what my biology teacher once referred to as *in silico* experiments?

Albert: No, not at all. Computer simulation is often referred to as 'computer experiments' or, in analogy with *in vivo* and *in vitro* experiments, also called *in silico* experiments because of the close analogies between laboratory experiments and computer simulation. Laboratory experiments are performed on a sample of a material whereas computer simulation is an experiment with models. The computer program is the analogue of the experimentalist's apparatus, testing of a program with known and well understood models is the analogue of the calibration of the apparatus in laboratory. Computation is the analogue of experimental measurement and, finally, both laboratory experiments and computer experiments require data analysis.

Laboratory experiments are performed on a sample of a material whereas computer simulation is an experiment with models.

Alice: Can we simulate any arbitrary molecular machine with a computer?

Albert: No, there are practical limitations. The main difficulties faced in computer simulations arise from the limited size of the available computer memory and the limitations imposed by the available computer time.

Alex: Could you please tell me what are the various types of molecular machines that operate inside an eukaryotic cell?

Albert: First, we can group the machines into two categories: one-shot machines and cyclic machines. The one-shot machines convert one form of energy (usually, chemical energy) into another (usually mechanical energy) only once, often in a sudden burst. In contrast, the machines belonging to the other group work in cycles just like engines of our automobiles. The cyclic machines, in turn, can be classified into different cate-

Computation is the analogue of experimental measurement and, finally, both laboratory experiments and computer experiments require data analysis.

Motor	Track
Kinesin	Microtubule
Dynein	Microtubule
Myosin	Actin filament
DNA helicase	DNA strand
RNA helicase	RNA strand
DNA polymerase	DNA strand
RNA polymerase	DNA strand
Ribosome	RNA strand

Table 1. Some common molecular motors and the corresponding tracks.

gories – motors, pumps, etc. The motors can also be divided into two categories, namely, linear and rotary; the linear motors move along filamentary tracks consuming fuel whereas rotary motors are like Alice's hair dryer.

Alex: Could you please give me examples of filamentary tracks used by the molecular motors?

Albert: Microtubules and flilamentary actin are most common tracks made of proteins. Nucleic acids like, for example, single-stranded DNA and mRNA serve as tracks for polymerases and ribosomes, respectively (see *Table* 1).

Alice: I have seen labourers laying down the railway tracks. Who plan and construct the network of the tracks inside the cells for the molecular motors?

Albert: In contrast to the railway tracks, the tracks for the molecular motors are dynamic.

Alice: Could you kindly give some examples of such dynamic tracks?

Albert: Microtubules, for example, are known to exhibit an unusual polymerization-depolymerization dynamics even in the absence of motor proteins. Moreover, in some circumstances, the motor proteins interact with the microtubule tracks so as to influence their length as well as shape; one such situation arises during cell division (the process is called *mitosis*). Trains never create their track. But, a DNA helicase motor unwinds a double-stranded DNA and uses one of the single strands thus opened as the track for its own movement.

Microtubules and flilamentary actin are most common tracks made of proteins. Nucleic acids like, for example, single-stranded DNA and mRNA serve as tracks for polymerases and ribosomes.

Alice: Wow! Do you identify the helicases, polymerases and ribosomes as motors just because they move on DNA or mRNA strands?

Albert: No doubt, they can be treated as molecular mo-

tors although, unlike cytoskeletal motors, they do not carry cargo. However, from a different perspective, a helicase is a "unzipper", a polymerase is a "copying machine" and the ribosomes are "assembly lines". In many circumstances, polymerizing microtubules and actin filaments act like nano-pistons. There are also examples of clamps and latches in eukaryotic cells.

Alex: Can one think of some other ways of classifying these molecular machines?

Albert: Sometimes the molecular machines are classified according to the environment where they operate. The cytoskeleton-based machines are constituents of the cytoskeleton or motors that move on cytoskeletal filaments. The helicases, polymerases and ribosomes are nucleic acid based motors whereas pumps, protein translocation machines, ATP synthase and flagellar motors are associated with membranes.

Alice: The machines which carry cargo are more like 'porters' who carry luggage on their head; unlike your car, these machines do not move on wheels.

Albert: You are right. First of all, none of the molecular motors in our cell has any part which even remotely resembles a wheel. In fact, there have been lively debates on this topic [4, 5] and it has been argued that in the soft world of living systems, it may be advantageous to have wheel-less transporters. Moreover, some authors have classified cytoskeletal motors into two groups: 'porters' and 'rowers'. Kinesins and dyneins are examples of cargo-carrying porters whereas myosins are typical examples of rowers.

Alice: Are you drawing any analogy between the myosins and the rowers whose rythmic movement of oars in and out of water impart high speed to the boats?

Albert: Yes, indeed. There are close similarities between

Trains never create their track. But, a DNA helicase motor unwinds a double-stranded DNA and uses one of the single strands thus opened as the track for its own movement.

None of the molecular motors in our cell has any part which even remotely resembles a wheel. It has been argued that in the soft world of living systems, it may be advantageous to have wheel-less transporters.

the two situations. Each of the oars spend little time under water in each cycle, but the collective push of the rowers act constructively leading to the high speed of the boat. Similarly, each myosin spends only a small fraction of its biochemical cycle in contact with the actin track exerting a tiny force, but the collective action of a large number of the mysosin motors generates forces large enough for muscle contraction.

Alice: Isn't the mechanism of molecular machines very similar to those of their macroscopic counterparts?

Albert: 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_BT, respectively (k_B being the Boltzmann constant). From a comparison of some of the characteristic features of the molecular motors and macroscopic motors (see *Table* 2), naively, at first sight, one may think that the differences in the mechanisms of the molecular and macroscopic machines is merely a matter of two different scales (of size, time, force, energy, etc.). But, that is not true. Since the masses of the molecular machines are extremely small, they are subjected to two dominating forces which are quite small for the macroscopic machines.

Alex: Does it mean that the molecular machines are not governed by Newton's law?

Table 2. Comparison of some of the features of macroscopic and molecular machines.

Feature	Macroscopic motors	Molecular motors
Material	Mostly Hard matter	Mostly Soft matter
Motor track	Road or rail	Filamentary proteins or nucleic acids
Fuel	Mostly petrol or diesel	Mostly ATP or GTP
Directionality	Bidirectional	Unidirectional
Speed	~1 Km/min.	~15 microns/sec
Force	~1000 N	~pN
Energy	1-100 J	1-100 $\times$ 10^{-21} J

Albert: Yes and no. The equation governing the motion of the machines is still Newton's equation, namely, mass $\times$ acceleration = force. But, there are crucial subtleties here. Because of its tiny mass, the inertial forces are orders of magnitude smaller than the viscous forces it experiences in the aqueous medium; in technical terms, one says that the dynamics of molecular motors is dominated by hydrodynamics at low Reynold's number [6].

The dynamics of molecular motors is dominated by hydrodynamics at low Reynold's number.

Alice: What is the Reynold's number?

Albert: It is the ratio of the inertial and viscous forces. It can be written as $\mathcal{R} = \rho L v/\eta$ where L is a characteristic linear size of the object, ρ and η are, respectively, the density and viscosity of the liquid and v is the relative velocity of the object with respect to the liquid. For proteins in aqueous solution, $L \simeq 10nm$, $\rho \simeq 10^3 Kg/m^3$, $\eta \simeq 10^{-3} Pas$, $v \simeq 1m/s$ (corresponding to $1nm/ns$). Consequently, the corresponding Reynold's number is $\mathcal{R} \simeq 10^{-2}$ (and even smaller for slower motions) whereas when you swim $\mathcal{R}$ is of the order of 10^4.

Alice: What difference does it make physically?

Albert: In order to appreciate the difficulties of swimming at low Reynold's number, let us multiply L by a factor of 10^7, without altering ρ and v, so that η must be multiplied by 10^7 to keep R unaltered. In other words, if a motor tried to swim in an aqueous medium adopting the swimming styles of humans, the difficulties it would face will be very similar to those that you and I would face if we ever tried to swim in honey. Interestingly, because of the low Reynold's number, the nano-motors can come to a halt practically instantaneously as soon as they apply on the brake.

If a motor tried to swim in an aqueous medium adopting the swimming styles of humans, the difficulties it would face will be very similar to those that you and I would face if we ever tried to swim in honey.

Alex: Is that the only difference between molecular and macroscopic machines?

Albert: No. The molecular machines get bombarded

The molecular machines get bombarded from all sides by the randomly moving water molecules. Because of these bombardments, the machines experience an additional force which is totally random.

from all sides by the randomly moving water molecules. Because of these bombardments, the machines experience an additional force which is totally random. The masses of molecular machines are so small that these *random thermal forces* have a strong infuence on their movement. It is because of these random forces that the molecular motors inside the cell move in a manner that resembles the movement of a human being in a storm.

Alice: I do not have any feeling for the level of difficulty faced by the motors because of this storm-like situation. Could you kindly give us some concrete idea?

Albert: Let me consider sensory neurons, i.e., some special types of cells of your brain. If all the lengths are multiplied by 10^6, then the transport from the cell center to the cell periphery is comparable to transportation of cargoes, which are supplied by a chemical plant of 10 meter diameter, for more than 300 kilometers along an approximately cylindrical pipe whose diameter is no more than 3 meters. This cargo transport is to be achieved even when the transport vehicles are getting bombarded from all sides by a violent storm!

Alice: What is meant by a 'random force'?

Albert: Well, even if you know the force acting on the machine at this instant of time, you have absolutely no idea of its magnitude and direction at any later time.

Alex: Then, how does one integrate the Newton's equation for the machine and find out its trajectory?

Albert: Very good question, Alex. In fact, the machine does not have any unique trajectory for a given initial condition; if you repeat the experiment, starting from identical initial conditions, the trajectories will be different! In other words, the equation of motion for the molecular machine (for simplicity, in one dimensional

space) is given by

$$0 \times \frac{d^2x}{dt^2} = F_{load} - \gamma \frac{dx}{dt} + F_{br},$$

where the first term on the right hand side denotes the externally applied load force while the viscous drag force, captured through the second term on the right hand side, is assumed to be proportional to the velocity of the motor. The last term in this equation represents the random Brownian force.

Alex: Do you mean to say that the molecular motor behaves as a Brownian particle?

Albert: Yes, almost. However, unlike passive Brownian particles (like, for example, pollen grains in water), the molecular machines need (free-) energy input [7] and, therefore, these are often regarded as 'active' Brownian particles. At least some of these machines are physical realizations of Brownian ratchet, a device that exhibits directed, albeit noisy, movement in spite of being subjected to random Brownian forces as they operate in conditions far from thermodynamic equilibrium [7-9].

Alex: Is this relation with Brownian motion and non-equilibrium processes motivating many statistical physicists like you to get into this area of research?

Albert: These are certainly motivating many statistical physicists to work on biological machines. There are also other related motivating factors. The durable parts of macroscopic machines are normally 'hard' so as to survive the regular wear and tear. In contrast, the structural elements of the cell, e.g., filaments and membranes, are 'soft'. These materials can be deformed easily because their conformations are determined by weak forces like, for example, van der Waals forces, hydrogen bonding, etc. Moreover, since the thermal energy k_BT available at a temperature T is comparable to these weaker forces, thermal fluctuations can also lead

The durable parts of macroscopic machines are normally 'hard' so as to survive the regular wear and tear. In contrast, the structural elements of the cell, e.g., filaments and membranes, are 'soft'.

Not only statistical physicists like me, but also physicists who have been working for many years on soft matter are getting interested in soft bio-materials.

to conformational changes. Furthermore, straightening of a filament or flattening of a membrane reduces its entropy and the corresponding restoring force is of entropic origin. Therefore, the free energies of soft matter are often dominated by entropy which gives rise to exotic phenomena that are not observed in hard materials. Naturally, not only statistical physicists like me, but also physicists who have been working for many years on soft matter are getting interested in soft bio-materials.

The level of the discussion was now too high for Alice. She was watching the neighbour cleaning his car and thinking about the rising prices of petrol and cooking gas.

Alice: I could not understand how ATP molecules serve as fuels for molecular machines.

Albert: ATP is the energy currency of the cell and is composed of a sugar-base-phosphate combination found in nucleotides; the sugar of this energy currency is ribose, the base is adenine. Adenosine triphosphate (ATP) has higher energy than adenosine diphosphate (ADP) and, the process whereby ATP gets converted to ADP and inorganic phosphate is called hydrolysis. The energy released by each ATP molecule through this process is about 10^{-19} J which corresponds to about $20k_BT$ at room temperature. The reverse energy consuming process, through which the spent fuel is recharged, is called phosphorylation. Just as Euro is used in many

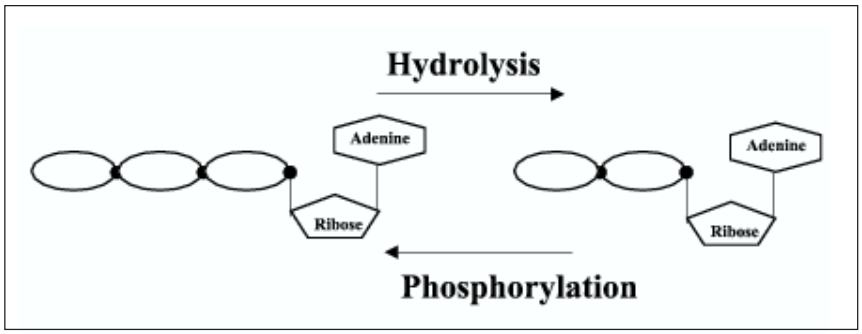

Figure 2. Schematic representation of ATP and its hydrolysis.

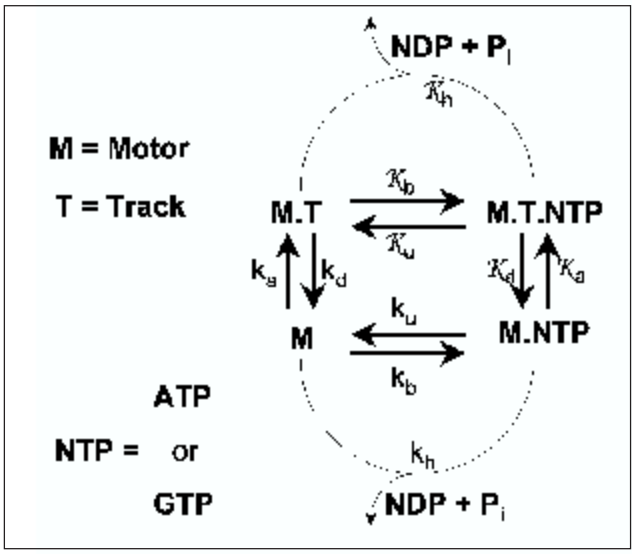

Figure 3. Biochemical cycle in the absence and presence of the track.

places as the international currency, instead of US Dollar, some molecular machines use guanine triphosphate (GTP), instead of ATP, as the energy currency.

Alex: Does the presence of the track affect the rate of hydrolysis of ATP by the motor?

Albert: Yes, indeed. The motor proteins work as enzymes even in the absence of the track. However, their enzymatic activity is boosted when the motor is bound to its track.

Alex: Is this enhanced enzymatic activity of a motor in the presence of its track related to its walking ability along the track?

The motor proteins work as enzymes even in the absence of the track. However, their enzymatic activity is boosted when the motor is bound to its track.

Albert: In the presence of the filamentary track, the mechanical and chemical cycles of the motor get coupled. For example, in case of conventional myosin, a mechanical step (the binding of the motor with the actin filament) catalyzes a chemical step (the release of phosphate) and, then, a chemical step (the binding of ATP) catalyzes a mechanical step (the detachment of the motor from the actin filament). Similarly, in case of conventional double-headed kinesin, a chemical step (the

The diffusion current along the mechanical coordinate is the velocity of the motor in real space, whereas that along the chemical coordinate is a measure of the enzymatic turnover rate.

binding of ATP to head 1) catalyzes a mechanical step (the attachment of the head 2 to the MT) which, in turn, catalyzes a chemical step (the release of ADP from head 2) which, in turn, catalyzes a mechanical step (the detachment of head 1 from the MT) (see, for example,[7] of Part 1). Thus, at the most abstract level, the problem of a molecular machine can be formulated as that of *mechano-chemistry* [10].

Alex: Could you kindly give me some intuitive physical picture of the *mechano-chemistry* of molecular motors?

Albert: Suppose, the system is being described by y_1, y_2, which represent the mechanical and chemical variables, respectively. The free energy $G(y_1, y_2)$ can be represented by a landscape such that $G(y_1, y_2)$ is the height (or, depth) of the landscape at the location y_1, y_2. In this scenario, the diffusion current along the mechanical coordinate is the velocity of the motor in real space, whereas that along the chemical coordinate is a measure of the enzymatic turnover rate. Thus, the landscape, i.e., the free-energy surface determines the kinetic mechanism of the motor [11, 12].

Alex: I guess the landscape shows periodicity along y_1 because the positions of the binding sites for the motor are arranged periodically on its track. Am I right?

Alex: Yes, in case of cytoskeletal motors the perodicity of the MT and actin tracks give rise to periodicity of the landscape along y_1 for any arbitrary fixed value of y_2.

Alex: Is the landscape periodic also in the y_2 direction?

Albert: Along y_2, the free energy diagram looks exactly like a typical free energy diagram for a chemical reaction where the two minima corresponding to the reactants and products are separated by a free energy barrier. Since the enzyme returns to its initial state after each enzymatic cycle, G is periodic also in y_2 except

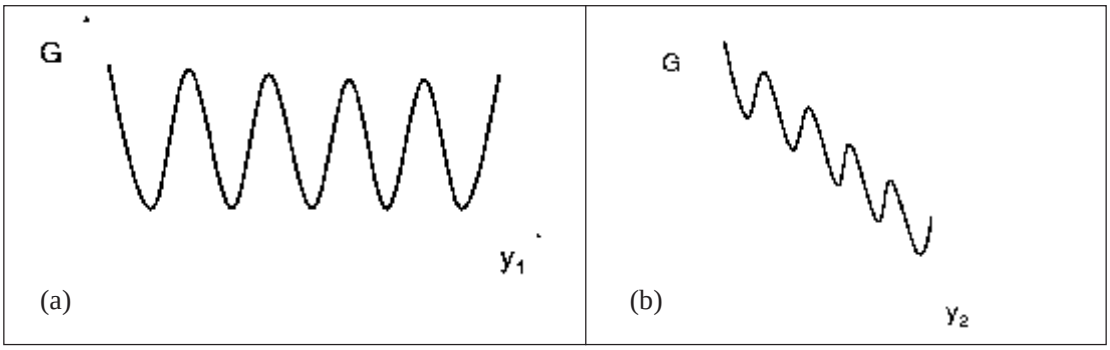

Figure 4. Two different cross sections of the free energy landscape. Variation of the free energy G with the 'mechanical variable' y_1 and chemical variable y_2 are shown in (a) and (b), respectively.

for an additional term that accounts for the free energy released by the reaction. In other words,

$$G(y_1 + d, y_2) = G(y_1, y_2)$$
$$G(y_1, y_2 + \Delta y_2) = G(y_1, y_2) + \Delta G, \qquad (1)$$

where d is the spatial period along y_1 (i.e., spacing between the successive binding sites on the track) while Δy_2 is the periodicity in the chemical variable.

Alice: Is it possible to visualize on the free-energy landscape the typical route of a motor?

Albert: Yes. In general, the landscape will have *local minima* connected by *low-energy passes*. These passes together define a low-energy path. The projection of the path on the $y_1 - y_2$ plane is neither parallel to y_1 nor parallel to y_2. Consequently, the tilt of the landscape in the chemical direction drives the mechanical movement of the motor utilizing the free energy released the by chemical reaction to do the necessary mechanical work.

Alex: What kind of fundamental questions are addressed in this area of interdisciplinary research?

Albert: Well, some of the fundamental questions are:

(i) What are the *moving parts* of the motor and what are their molecular constituents?
(ii) What is the *fuel* that supplies the (free-)energy input for the machine ?

(iii) How does the *transduction* of energy take place?
(iv) What is the most appropriate definition of efficiency of the motor and how to estimate that efficiency?
(v) How is the operation of the machine *regulated* and *controlled*? For example, how is the machine switched on and off?

In addition several other fundamental questions are addressed in the context of specific molecular machines.

Alice: Is this research important from a biomedical perspective? Can the results of these investigations improve the quality of human life?

Albert: Yes. First, it is not surprising that defective molecular machines can cause diseases [13, 14] just as occasional disruption of work in any department of a factory can bring the entire factory to a standstill. In fact, malfunctioning of the track and/or motor can cause breakdown of the intracellular molecular motor transport system. Moreover, viruses are known to hijack the motors to travel from the cell periphery to the cell nucleus. Therefore, if we understand how motors work at the molecular level we will not only be able to fix them when they malfunction but also control their progress and even find cures for the disease. For example, we might also devise ways to selectively either arrest subcellular processes that cause diseases like cancer or slow down metabolism of organisms that invade cells causing other types of disease. The molecular motors can be used as vehicles for drug delivery [15]. Thus, research on molecular machines can contribute towards improvement of human health and fitness.

If we understand how motors work at the molecular level we will not only be able to fix them when they malfunction but also control their progress and even find cures for the disease.

Alice: I am so impressed by Mother Nature! I wish our factories were as sophisticated as the cell.

Albert: Alice, you may be glad to know that a large number of experts working in the area of nano-technology are drawing lessons from the principles used by nature

in designing the molecular motors with the aim of synthesizing artificial nano-machines [16].

Alex: Are all the physicists and engineers following the same approach in nano-technology?

Albert: No. 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, the natural molecular machines opened up a new frontier of nano-technology and provides an alternative approach – the bottom-up (from smaller to larger) approach [16]. This approach is pursued mostly by chemists. Feynman, a great visionary, made the prophetic statement in his famous talk at the American Physical Society [3]: "ultimately, we can do chemical synthesis... The chemist does a mysterious thing when he wants to make a molecule. He ....mixes this and that, and he takes it, and he fiddles around. And, at the end of a difficult process, he usually does succeed in synthesizing what he wants". Indeed, in recent years, several artificial molecular machines have been synthesized chemically [16]. In order to get some lessons from Nature's billion year experience in nanotechnolgy, we must carry out reverse engineering.

The natural molecular machines opened up a new frontier of nano-technology and provide an alternative approach – the bottom-up approach.

Alice: What is reverse engineering?

Albert: Reverse engineering is "the process of analyzing a system to identify the system's components and their interrelationships and create representations of the system in another form or at a higher level of abstraction" [17]. Reverse engineering of natural nanomachines will give us clues as to the possible design principles that can be utilized to synthesize artificial nano-machines. In fact, the term biomimetics has become a popular buzzword; this field deals with the design of artificial materials utilizing the principles of biomaterials [18].

Biomolecular nano-machines have become a subject of truly interdisciplinary research that involves biology, chemistry, physics as well as engineering and technology.

Albert: To summarize what has emerged from our long discussion, it has become clear that the cell is more like a factory where active biological processes are driven by coordinated operation of a large variety of molecular machines whose design has been perfectd by millions of years of evolution. One of the challenges of fundamental research is to understand the mechanisms of their operation in terms of the physical principles governing their structure and dynamics. The results of these investigations are likely to have significant impact on applied research in biomedical sciences and have opened up novel approaches in nano-technology. Biomolecular nano-machines have become a subject of truly interdisciplinary research that involves biology, chemistry, physics as well as engineering and technology.

Acknowledgements

Indrani Chowdhury holds copyright of the figures; I thank her for giving me the required permission to reproduce the figures in this article. I also thank Aakash Basu and Debanjan Chowdhury for critical reading of the manuscript.

Suggested Reading

[1] J M Imhof and D A Vanden Bout, *Am. J. Phys.*, Vol. 71, p.429, 2003.
[2] E Frey, *ChemPhysChem,* Vol. 3, p.270, 2002.
[3] R P Feynman 1959, included also in: *The Pleasure of Finding Things Out*, Perseus Books, Cambridge, Massachusetts, chapter 5, 1999.
[4] S J Gould, *Hen's Teeth and Horse's Toes*, W W Norton and Co., 1984.
[5] R Dawkins, *The Sunday Times*, Nov.24th, 1996.
[6] E M Purcell, *Am. J. Phys.*, Vol.45, p.3, 1977.
[7] D Chowdhury, *Resonance,* Vol.10, No.9, p.63, 2005; Vol.10, No.11, p.42, 2005.
[8] P Reimann, *Phys. Rep.*, Vol.361, p.57, 2002.
[9] F Julicher, A Ajdari and J Prost, *Rev. Mod. Phys.*, Vol.69, p.1269, 1997.
[10] C Bustamante, Y R Chemla, N R Forde and D Izhaky, *Annu. Rev. Biochem.*, Vol.73, p.705, 2004.
[11] D Keller and C Bustamante, *Biophys. J.*, Vol.78, p.541, 2000.
[12] C Bustamante, D Keller and G Oster, *Acc. Chem. Res.*, Vol.34, p.412, 2001.
[13] M Aridor and L A Hannan, *Traffic*, Vol.1, p.836, 2000; Vol.3, p.781, 2002.
[14] N Hirokawa and R Takemura, *Trends Biochem. Sci.,* Vol.28, p.558, 2003.
[15] R N Cohen, M J Rashkin, X Wen and F C Szoka Jr., *Drug Discovery Today: Technologies,* Vol.2, p.111, 2005.
[16] V Balzani, M Venturi and A Credi, *Molecular Devices and Machines: a Journey into the Nano-world*, Wiley-VCH, 2003.
[17] E J Chikofsky and J H Cross, *II. IEEE Software,* Vol.7, pp.13–17, 1990.
[18] Y Bar-Cohen, ed., *Biomimetics: Biologically Inspired Technologies*, Taylor and Francies, 2005.

Address for Correspondence
Debashish Chowdhury
Department of Physics
Indian Institute of Technology
Kanpur 208016, India.
Email:debch@iitk.ac.in