

B 10 Biological Nanomachines

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1 Introduction

Nanotechnology is perfectly realized in biological systems. Cells are essentially biological assemblers that build thousands of custom-designed molecules and construct new assemblers. This view [1] was pioneered by Richard Feynman [2] and popularized by Eric Drexler's [3, 4] evocative idea of a self-replicating assembler building nanoscale devices atom by atom. Bio-nanotechnology, being a synonym for nanobiotechnology, is a rapidly growing field that encompasses contributions from various disciplines, ranging from engineering and computational sciences to physics, chemistry and biology.

It is now clear that most functions in the cell are not carried out by single protein enzymes, colliding randomly within the cellular jungle, but by macromolecular complexes containing multiple subunits with specific functions [5]. Living cells are made up of these complexes, which carry out many of the functions essential for their existence, differentiation, and reproduction. In many cases the malfunction of these proteins can be a source of disease; for example, myosin mutations, particularly in the head and neck region of the molecule, can result in inherited diseases such as familial hypertrophic cardiomyopathy. An understanding of the mechanisms of these proteins may provide a guide for therapy.

Many of these complexes can be described as "molecular machines" or "molecular motors" or "molecular devices", depending on their sizes, complexity and tasks [6]. Indeed, this designation captures many of the aspects characterizing these biological complexes : modularity, complexity, cyclic function, and, in most cases, the consumption of energy. In molecular machines or motors, a rotary or linear movement is used for motility, nucleic acid processing, folding or unfolding, or as transducers of light or chemical energy. Examples of such molecular machines are the replisome, the transcriptional machinery, the spliceosome, the ribosome, but also smaller machines, residing in the plasma and organellar membranes, are known as ion and protein transporters, or as the bacterial flagellar motor.

The size of these macromolecular complexes, alone, often makes them inaccessible to x-ray crystallography. The structure determination of large, complex protein ensembles will pose a particularly difficult problem for structural biologists. As a result, many efforts have concentrated on determining the structures of individual subunits and domains within the machines. Consequently, information on the organization of the assembly subunits, their interactions, and sometimes their precise function within the context of the fully functional complex is often lost.

In the post-genomic era, the recognition that computational methodologies will play a critical role in biology is widespread. Cell and molecular biologists have realized the impracticality of trying to successfully predict complex molecular mechanisms using intuition. Accordingly, the molecular and cell biology communities are now seeking suitable avenues for enabling them to add computational tools to their research kits.

The essential question in understanding biomolecular machines is concerned with the explanation of the macroscopic phenomenology in terms of the atomic structures and forces involved. Although a complete description is not yet available even for the best-characterized system considerable progress has been made recently, not only from an experimental point of view, but also with respect to computational and theoretical achievements. Some of these machines, which have been studied by simulations and mathematical methods are listed in Table I (section 4).

2 Molecular Models : Force Fields, Water, Proteins, Lipids

It is generally believed that biological systems can be fully described by the Schrödinger equation:

$$i\hbar \frac{\partial \Psi}{\partial t} = -\frac{\hbar^2}{2} \sum_{a=1}^N \left(\frac{\nabla_a^2}{m_a} \right) \Psi + U(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N) \Psi \quad (1)$$

where $U(\mathbf{r}_1, \mathbf{r}_2, \dots, \mathbf{r}_N)$ is the potential function of the N interacting particles, and Ψ is the wave function which describes the state of the system.

However, even when the approximations (e.g., Born-Oppenheimer [7], LDA [8,9], GGA [10]) have been applied and elegant methods (e.g. DFT [11]-MD in the scheme of Car & Parrinello [12]) have been employed, the current quantum mechanical studies are still limited by the time scale of few picoseconds and the length scale of some tens of Å.

It is no doubt that biological systems are full of complexity. Typical characteristic time scales are very long, ranged from subnanosecond (e.g., passage of water or ions through pores or channels [13]) to minutes (e.g., resealing of pores in the electroporation experiments [14, 15]). It is thus necessary to employ even more crude descriptions for the systems of interest. Before doing that, we first look at some important aspects of biological systems.

2.1 Force Fields

Finally we come to the approach we take for modeling biological macromolecules, the so-called *force field* approach. A force field is a mathematical function, which returns energy as a function of *conformations*, or *atomic coordinates*. Here the electronic degrees of freedom are totally neglected. Typically, force fields are sums of terms which correspond to stretching, bending, torsion, van der Waals and electrostatic interaction energies as functions of conformations:

$$U(\mathbf{q})_{total} = U_{stretching} + U_{bending} + U_{torsion} + U_{vdw} + U_{electrostatic} \quad (2)$$

where $\mathbf{q} = (q_1, q_2, \dots, q_{3N})$ represents the conformation of the model system and N is number of atoms in the system. Most commonly, the terms are summations of the following form:

$$\begin{aligned} U(\mathbf{q})_{total} = & \sum_{\text{bonds}} K_r (r - r_{eq})^2 + \sum_{\text{angles}} K_\theta (\theta - \theta_{eq})^2 + \sum_{\text{dihedrals}} \frac{V_n}{2} [1 + \cos(n\phi - \gamma)] \\ & + \sum_{i < j} \left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^6} + \frac{q_i q_j}{\epsilon R_{ij}} \right] + \sum_{\text{H-bonds}} \left[\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}} \right] \end{aligned} \quad (3)$$

The stretching, bending, torsion and improper torsion terms are summed over all bonds, angles, torsions and improper torsions, respectively. The van der Waals and electrostatic terms are summed over all possible pairs of atoms. The main features of the van der Waals interactions are the short repulsion and the long attraction, which is usually modeled by the 6-12 Leonard-Jones potential for non-hydrogen-bonding pairs. 10-12 terms are used in modeling hydrogen bonds for two main reasons. First, during the energy refinement, it was found that stronger repulsion terms are required to prevent the occurrence of unrealistic short hydrogen bonds. Second, 10-12 functions allows one to “fine tune” the hydrogen bonds to desired values. However, in some of the new force fields the 10-12 terms have been abandoned.

Molecules are represented in force fields as a collection of charged point masses which correspond to atomic centers. In the 1980's, hydrogens were typically not represented explicitly for the sake of computational speed.

In summary, broadly speaking, there are three steps in molecular modeling:

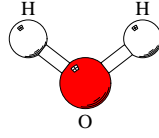
1. Find the structure (atomic coordinates) of the molecules of interest.
2. Establish the topology to the molecules and assign atom types on each atom. This is where trouble can occur without being detected. Special care should be taken in this step.
3. The molecular structural energy can now be evaluated with the use of a force field or potential function. Energy will be evaluated repetitively during MD, MC or minimization procedures as the conformation is altered.

We have to emphasize that the force field approach is a sophisticated though still imperfect way for describing the interactions of molecules. In principle, it is possible to derive all the components of a force field from quantum mechanical calculations. A continuous "Born-Oppenheimer surface" would provide detailed informations on the energetic cost of conformational transitions. Analysis, in turn would lead to force constants and equilibrium values for all terms. However, in practice, this is possible for only a few hundreds of atoms. The basis of a force field is the choice of **atom types**; i.e., the selection of atoms which are enough alike, both chemically and physically, to be treated identically in the molecular mechanics refinement. The decisions on atom types are inevitably compromises between the most accurate representation of many molecules and have manageable number of types. In AMBER, for instance, atom type 'C' is employed for carbon in carbonyls while atom type 'CA' is employed for carbon in benzene and benzene derivatives. Bond, angle, torsion parameters usually come from empirical sources and are referred as **bonded terms**. Equilibrium bond, angle and torsion values are obtainable from analysis of crystallographic databases of small molecules. The Cambridge Database is a repository of such data. Allen *et al.* [16] presents a detailed analysis of the geometry of the common organic functional groups. This type of data is the common source of equilibrium parameters. Force constants are most commonly derived from infrared spectra, which is naturally well suited to describing force constants.

Nonbonded terms are mainly two parts: van der Waal interactions and electrostatic interactions. Nonbonded interaction are the most difficult part for force field parameterization. Van der Waals parameters can likewise be derived from analysis of x-ray and neutron diffraction data.

Van der Waal terms, along with the above bonded terms, are from the geometric properties of molecules. The strictly geometric properties are well understood and well described by force fields. The geometric terms do not vary a great deal from force field to force field since they are all derived from the same common pool of experimental data. Unfortunately, the geometric terms are only a minor consideration compared to the electrostatic interactions which are not so well described.

Electrostatic terms are dictated by the assignment of charges on the atoms. There are at least two problems with assignment of charges: 1. experimental data of charges for molecules at atomic level resolution does not exist. 2. the basic underlying molecular model used with force fields is a truncation of what a molecule is. Molecules are collections of nuclei which share common electrons. The nuclei have charges equal to the sum of their protons. Each electron has a (-1) charge and cover the nuclei in electronic clouds. The exact localization



(a)

Fig. 1: Schematic representation of the TIP3P water model.

of the electrons can change slightly depending on environment, and this feature is called the *polarizability*. In force fields, molecules are mostly represented as a collection of fixed charged point masses. Assigning charges to these point masses is not a completely resolved issue. Charge assignment methodology differs for the programs GROMOS [17, 18], CHARMM [19] and AMBER [20, 21].

In summary, geometric constraints are derived largely from experimental data. These terms which describe bond, angle and torsion lengths and vibrations are well understood and well described in force fields. Likewise, van der Waal terms are well understood and described in force field. Unfortunately, the term that really counts (generally by one or two orders of magnitude) is the poorly understood and described electrostatic term. Charges are derived by a variety of ways depending on force field developers. In the following sections a short introductions to the modeling of the elements in membrane protein interaction studies, namely, water, proteins and lipids, are presented.

2.2 Water

Water is one the most mysterious substances on the earth. Without water, there would be no life. Water is indispensable in all biological systems, and the modeling of the water molecule serves as the first step for all the biological molecules. Modeling of water can be traced back to the pioneering work of Bernal and Fowler in 1933 [22]. The first computer simulation of liquid water was performed with the Monte Carlo method by Barker and Watts in 1969 [23] and then followed by Rahman and Stillinger in 1971 [24] with the molecular dynamics method. For rigid molecular models the potential for two water molecules i and j , u_{ij} , is usually in the following form:

$$u_{ij} = \sum_{c,c'} \frac{q_c q_{c'}}{r_{cc'}} + \frac{A}{r_{OO'}^{12}} - \frac{C}{r_{OO'}^6} \quad (4)$$

$$= \sum_{c,c'} \frac{q_c q_{c'}}{r_{cc'}} + 4\epsilon \left[\left(\frac{\sigma}{r_{OO'}} \right)^{12} - \left(\frac{\sigma}{r_{OO'}} \right)^6 \right] \quad (5)$$

where $q_c, q_{c'}$ are charges on molecule i and j respectively, $r_{cc'}$ is the distance between two charges, $r_{OO'}$ is the distance between two oxygen atoms in molecule i and j . The parameters and geometries for various rigid water models (SPC [25], TIP3P [26], TIP4P [26], SPC/E [27], WK [28]) differ to some extent. As an example, the TIP3P model [26] has the following parameter as given in Table 1. In all the models the sites for Lennard-Jones interactions locate at

$r(\text{OH})$ (Å)	$\angle(\text{HOH})(^{\circ})$	$10^{-6} A$ (kJ Å ¹² mol ⁻¹)	$10^{-3} C$ (kJ Å ⁶ mol ⁻¹)	(O) ($ e $)	(H) ($ e $)
0.9572	104.52	2.633	2.617	0.834	0.417

Table 1: Parameters for TIP3P rigid 3-charge water model [26].

the oxygen atoms, however, we can see that for some models (TIP4P, WK, etc.) the site of the negative charge is moved off the oxygen to a point 0.15 Å along the bisector of HOH angle. Evaluation of an intermolecular interaction requires computation of nine for three-site models or ten for four-site models.

2.3 Proteins

Proteins consist of amino acids, and sometimes are also called polypeptides. The ability of a polypeptide chain to fold up into a unique, highly ordered structure is the most important feature that distinguishes a biologically active protein from an inert polymer. For many proteins, especially small ones with a single globular domain, this remarkable *order-disorder* transition can be reproduced in the test-tube, making it accessible to direct physical investigation. Equilibrium studies where the interconversion between folded and unfolded states is observed as a function of extrinsic variables, e.g., temperature, solvent composition, pH, ionic strength, pressure, etc., have led to a thorough understanding of the thermodynamic properties of folded and unfolded proteins, and the application of site-directed mutagenesis has provided important clues to the interactions that stabilize the native structure. Different conformations of a protein differ only in the angle of rotation about the bonds of *backbone* and amino acid *side chains*, although conformations may also differ in their disulfide bonds. The ideal unfolded protein is the *random coil*, in which the rotation angle about each bond of the backbone and side chains is independent of that of bonds distant in the sequence and where all the conformations have comparable free energies, except when atoms of the polypeptide chain come into too close proximity. Steric repulsions between atoms close in the covalent structure place limitations on the local flexibility, and spatial overlap between atoms distant in the covalent structure would also exclude a fraction of totally random conformations (the “excluded volume effect”). The native, folded conformations of proteins are complex, but known in great detail from the structure determined by X-ray crystallography and NMR. The basic unit of protein folded structures is the *domain*, which has been defined in many different ways, but is basically a structural unit that could plausibly be imagined to be an independent structural unit and to remain folded in isolation. The interiors of domains consist primarily of elements of secondary structure (α -helices and β -sheets) packed together via their nonpolar side chains. The interactions determining the tertiary structure are largely between residues distant in the primary structure and hence known as ‘long range’ interactions. A variety of proteins have been observed under certain conditions to exist in stable conformations that are neither fully folded nor fully unfolded. These conformations are known as *molten globule* which is a metastable conformational state. The folded states of proteins are only marginally more stable than fully unfolded state, even under optimal conditions. Typically the free energy change for a small protein from an unfolded state to the native state is about -5 to -10 kcal/mol. In developing the force field parameters of the protein segments, the first criteria for the AMBER ’84 force field [29] was to derive a consistent set of charges for the hydrogen, nitrogen, carbon, and oxygen atoms contained in the amide segment of the peptide chain.

N-acetyl-*N'*-methylglycinamide and *N*-acetyl-*N'*-methylanalamide dipeptides were chosen in modeling the backbone of proteins.

2.4 Lipids

A lipid molecule can be subdivided into five groups : the head group, two ester groups, and two hydrocarbon chains. One may categorize lipids by their “spontaneous curvature”, i.e., the cur-

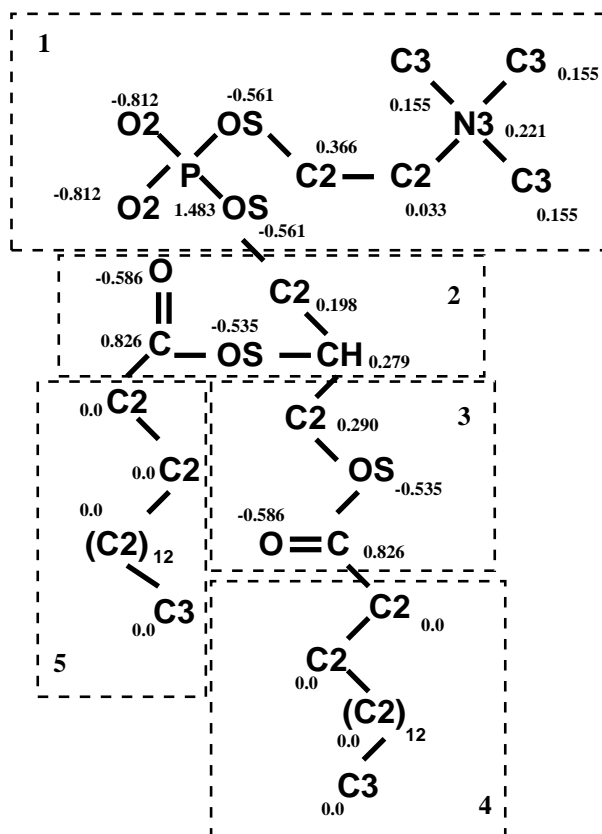


Fig. 2: Atomic charges and AMBER atom types of the DPPC molecule.

vature of a monolayer at a water-oil interface [30]. Bilayer forming lipids have a spontaneous curvature of zero; non-bilayer ones may have either positive or negative values. A significant fraction of cell lipids are non-bilayer-forming lipids. Previous studies established that such lipids facilitate fusion and transport by increasing membrane flexibility. Molecular descriptions of the structure and dynamics of membranes from either experimental or theoretical approaches are still lacking, partially due to the two-dimensional character of membranes, which makes difficult a detailed analysis by X-ray diffraction, neutron diffraction, or nuclear magnetic resonance. Force fields for united atoms provided by AMBER, CHARMM, and GROMOS were originally designed to describe proteins and nucleic acids, thus the application to membrane modeling is not straightforward. As an example, the configuration and charges of the DPPC (dipalmitoylphosphatidylcholine) lipid is given in Fig.2. Detailed derivations of an all-atom potential energy function for phospholipids within the CHARMM force field can be found in the articles by Schleckrich *et al.* [31] and Feller *et al.* [32].

3 Molecular Dynamics Simulation

A central problem for molecular simulations is the sampling of conformational degree of freedom. The two most widely used methods for atomic-level modeling of fluids are the Monte Carlo (MC) methods and the molecular dynamics (MD). Both procedures could use the same molecular models, classical force fields for the potential energy terms, and the implementation of boundary conditions. The principal differences are the modes of sampling the configuration space available to the system. The conventional form of molecular dynamics represents a realization of Boltzmann's approach to statistical mechanics, whereas the Monte Carlo method is rooted on the Gibbs' formulation of the problem. MC serves as a very robust algorithm and can be applied to more types of models and potential functions. The advantages of the MD methods are the efficiency [33–35] of searching in the phase space for high density systems and the built-in parallelizable nature. However, in some situations and by means of optimization of algorithms the MC methods could be still more efficient [35].

Introduction to Monte Carlo (MC) and molecular dynamics (MD) simulations are presented in details in the lectures presented by G. Vliegenhart and R. Winkler, respectively.

In following I will address only specific points related to MD simulations of membrane proteins. The molecular dynamics algorithms integrate the Newtonian equations of motion numerically. It is widely accepted that, to avoid instabilities, dissipation, and systematic drifts, the Hamiltonian must be conservative, and the integration method should be reversible and symplectic. The most common integrators, such as the Verlet leapfrog and velocity algorithms [36–38], satisfy these conditions, so that these problems are avoided, at least in principle, in the limit of very small timesteps.

Constraint Dynamics. For most of the flexible molecules that have been studied, the covalently bonded structure confines some of the internal coordinates to a narrow range of values at ordinary temperatures. Since the bond stretching frequencies are of the order of 30 ps^{-1} , and the bond angle bending frequencies are of the order of 10 ps^{-1} , root mean square (RMS) fluctuations of the order of 0.003 \AA for bond lengths and 5° for bond angles are expected. It has often been assumed that the bond lengths, the bond angles, and even some of the dihedral angles (e.g., peptide bond dihedral angles) can be treated as rigidly constrained. In this way the highest frequency components of molecular motions are eliminated and the size of the time step used in integrating the equation of motion can be increased. This makes it possible to obtain a threefold increase of the computational efficiency of macromolecular simulations.

To introduce necessary constraints, the SHAKE method [39, 40] is often used, which is especially appropriate for macromolecules since it treats the constraint one after another in an iterative way, therefore the increase in the required computer time is then proportional to the number of atoms N .

Extension from NVE to NPT Ensemble. For many years, the molecular dynamics (MD) method was limited to the microcanonical (N, V, E) ensemble in which the volume and the total energy are conserved. On the other hand, the Monte Carlo (MC) method was long ago extended from the normal canonical (N, V, T) ensemble to treat the constant pressure (N, P, T) and the grand canonical (μ, V, T) ensembles. It was Andersen [41] who first proposed the new constant pressure method (NMD) method in which the volume was allowed to fluctuate, its average value being determined by the balance between the internal stresses in a system and the external reference pressure. Andersen's method, which approximates a constant enthalpy-constant pressure (N, P, H) ensemble, introduces a parameter C , which determines the rate at which the volume fluctuates. At equilibrium, static quantities evaluated in this approximate ($N,$

P, H) ensemble are independent of the values. Parrinello and Rahman extended the method to allow the MD cell to change its *shape* and in this way they were able to explore the relationship between the interaction potentials and crystal structures [42]. It was demonstrated that Rb atoms initially disposed in a f.c.c. structure changed spontaneously to a b.c.c structure. Instead of modifying the Hamiltonian, Berendsen *et al.* [43] proposed a *weak coupling* approach to an external thermal bath or pressure bath, using the *principle of least local perturbation* consistent with the required global coupling. First consider the coupling of a system to a heat bath with fixed reference temperature T_0 . Such a coupling can be accomplished by inserting stochastic and friction terms in the equations of motion, yielding a Langevin equation:

$$m_i \dot{v}_i = F_i - m_i \gamma_i v_i + R_i(t) \quad (6)$$

where F_i is the systematic force and R_i is a Gaussian stochastic variable with zero mean and with correlation

$$\langle R_i(t) R_j(t + \tau) \rangle = 2m_i \gamma_i k_B T_0 \delta(\tau) \delta_{ij}. \quad (7)$$

where $\langle \rangle$ denotes the ensemble average. The friction coefficients γ_i determine the strength of the coupling of each atom to the bath. The coupling of temperature T to the heat bath of temperature T_0 can be related to :

$$\left(\frac{dT}{dt} \right)_{bath} = 2\gamma(T_0 - T) \quad (8)$$

$$= \frac{T_0 - T}{\tau_T} \quad (9)$$

where the time constant τ_T of this coupling is equal to $(2\gamma)^{-1}$. This leads to a rescaling of the velocities per time step in the algorithm from v to λv with (to the first order)

$$\lambda = 1 + \frac{\Delta t}{2\tau_T} \left(\frac{T_0}{T} - 1 \right). \quad (10)$$

Coupling to a constant pressure bath can be accomplished according to the same principle. In stead of modifying the Hamiltonian a very simple algorithm was proposed by Berendsen *et al.* [43] by adding an extra term to the equation of motion that effects a pressure change. The formula for box rescaling are :

$$\begin{aligned} \mu_x &= 1 - \frac{\beta_x \Delta t}{\tau_P} (P_{0x} - P_x) \\ \mu_y &= 1 - \frac{\beta_y \Delta t}{\tau_P} (P_{0y} - P_y) \\ \mu_z &= 1 - \frac{\beta_z \Delta t}{\tau_P} (P_{0z} - P_z) \end{aligned} \quad (11)$$

The compressibility, that may not be accurately known, occurs in the expression for the scaling factor μ . Since an inaccuracy in β only influences the accuracy of the noncritical time constant τ_P , the imprecision β is of no consequence for the dynamics. If β is not known for the potential model that is used in the simulation, it is sufficient to use an experimental value for the physical system that is approximated by the simulation.

4 Examples of biological nanomachines

Considerable progress has been made in the past two years by a combination of biophysical techniques and theoretical analysis in our understanding of a several biological machines, including single motor proteins (kinesin, myosin, polymerases) and various ATPase complexes, among the smallest biomolecular rotary motors.

Energy Source. The term ‘motor’ or ‘machine’ is used to describe some biomolecular complexes because they transduce one form of energy to another, e.g. chemical binding to mechanical work. These machines make use of chemical energy from a variety of sources, of which the most common is the binding energy of ATP, H_2O and its hydrolysis products ADP, H_2PO_4 : $ATP^{4-} + H_2O \rightarrow ADP^{3-} + H_2PO_4^-$. Proton and ion gradients, as well as redox potential differences, also serve as the energy source in some cases.

Structure. Biomolecular machines range from single subunits (e.g. DNA polymerases) through the smallest rotary motor, F_1 -ATPase, composed of nine subunits in mitochondria, to the flagella motor of bacteria, which can be composed of several hundred subunits of a number of different proteins.

Cellular Function. Each protein machine possesses its specific function and often it forms an element of the chemical network of which the cell is composed. The motors have a wide range of functions, including chemical (e.g. ATP) synthesis, organelle transport, muscle contraction, protein folding, and translocation along DNA/RNA and protein filaments. They play an important role in cellular signaling, cell division, and cellular motion.

Classification. Table I lists some of the known biomolecular machines with their functions and energy sources. There exists others and that all the functions of the known motors are not yet recognized. Table I includes the class of membrane channels. In a strict sense, these proteins are not motors, but rather ‘valves’ whose gating are controlled by some form of external free energy. However, since they are one of the most intensively studied biomolecular devices, they are discussed in a special subsection.

4.1 Membrane Channels

Membrane channels are proteins, which reside in membranes acting as ‘valves’ thereby permitting selective or non-selective flow of molecules along their concentration gradients across the membrane [44, 45]. Ion channels are highly specific filters regulating the ion balance of living cells. In contrast to transporters, channels are passive devices whose opening and closing (‘gating’) is controlled externally by ligands, voltage, pH value or mechanical stress.

A lot of experimental effort has been put into resolving membrane-bound processes, from the structural as well as dynamical point of view, but traditionally with a relatively small input from the theoretical approaches. As far as the molecular structure determination of membrane proteins is concerned, obtaining a crystallized functional form of the membrane protein is still a generally non-solved problem; up to now, only few structures have been resolved up to the atomic details. One of the recent major breakthroughs was the successful structural determination of the bacterial potassium channel KcsA [46–49], which have almost completed the detailed picture of structure-function relationship for the potassium ion transport process.

In the last few years, the most rapid development in the field of membrane proteins simulations has been seen specifically in the area of ion channels [50].

From a general point of view, voltage-gated ion channels are of particular interest. They control electrical activity in nerve, muscle and many other cell types. The voltage-gated ion channels,

Biomolecular Devices	Function	Energy source
Membrane Transducers		
GPCR Rhodopsin CCR5, CXCR4	signal relay	ligands
Catalytic Receptors Insulin, EGF	signal relay	photons, ligands
Chemotactic Sensors sR	signal relay	photons, ligands
Receptor-Ligand Titin, GroEL, etc.	signal relay unfolding, refolding	photons, ligands ATP
Membrane Channels		
Porins OmpF, OmpT, OmpA, FhuA AQP Maltoporin	valve for ion, water	gradients
Ion channels AChR CIC gA GluR KcsA KvAP MscL, MscS	selective ion valve	gating by... ligand $\Delta\Psi$ ligand pH voltage strain
Membrane Transporters		
Ion Translocons bR hR	ion pumps H^+ Cl^- , anion	photons
Ion translocases V-ATPase Ca^{2+} -ATPase Na^+ - K^+ -ATPase	ion pumps H^+ Ca^{2+} Na^+ , K^+	ATP ATP ATP
Solute Translocases HisP, MscA, GLUT3	solute transport	ATP
Protein translocases mitochondrial pore ER pore nuclear pore	protein transport	ATP ATP, $\Delta\Psi$ ATP, GTP
Membrane Motors		
ATP synthase Flagellar motor	ATP synthesis or H^+ pump bacterial motility	$\Delta\Psi$ or ATP $\Delta\Psi$
Motor Proteins		
Track Motors Myosin II Myosin V, VI Kinesin	muscle action cargo transport cargo transport	ATP ATP ATP
Dynein Nucleotidases Polymerase Helicase	flagellar motion catalyze synthesis unwind dDNA	ATP ATP ATP, n-phos ATP

Table 2: List of some molecular nanomachines which have been studied theoretically and by simulations. Abbreviations : ATP = hydrolysis of adenosine triphosphate, n-phos = nucleotide phosphorylation. $\Delta\Psi$ = membrane potential and ion gradient

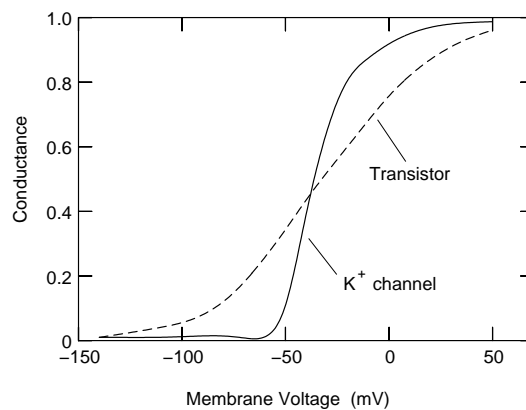


Fig. 3: *Voltage sensing in a potassium ion channel. The control of ion flow through voltage-gated channels is very sensitive to the voltage across the cell membrane. By comparison, an electronic device such as a transistor is much less sensitive to applied voltage.*

residing in membranes of living cells, from bacteria to humans, behave like field-effect transistors. In transistors, the flow of electrons through a semiconductor 'channel' is governed by the voltage applied to a 'gate' electrode. As voltage-sensing devices, these channels can perform much better than their electronic counterparts (Fig.3). Their high sensitivity to voltage is important, because cellular voltage changes are small.

In order to assure the precisely controlled transmembrane ionic flow, ion channels have to fulfill three specific points; i) high selectivity towards specific ion type (or have to highly discriminate one ion type over all the other ions present), ii) permeation at the diffusion rate (or to provide an energetically barrierless pathway which ensures the rapid transport of selected ions), and iii) well defined gating control mechanism, in which the conformation of the open state has to be accomplished upon specific stimulus; the known mechanisms include change in transmembrane potential (voltage-gated ion channels), binding of another molecule (ligand-gated or receptor-gated channels) and mechanical stress (mechanosensitive channels or stretch-gated channels).

While the timescale of the permeation process for the typical channel is in the order of few tenths of nanoseconds, the gating takes the time in the order of milliseconds. The present state of computational speed does not permit the completion of neither of the processes using MD techniques, calling for more coarse-grained modelling. On the other hand, BD and continuum theories do not distinguish between monovalent ions, so the modelling of channel selectivity appeals for the MD approach. Although Brownian dynamics (BD) simulations have provided useful data on the total permeation process of ions through channels [51], their reliability concerning details is limited due to the coarse-graining of the channel structure and applications of mean-field approximations of water and lipids. More details, including fluctuations of all constituents of the channel and its environment (all atom model of channel, water and lipids) have been elucidated by use of molecular dynamics (MD) simulations [50,52] albeit quite limited in time to the few nanoseconds time regime.

Permeation. The permeation process deals with an open state conformation of the protein; the challenge for the theoretical approach is to explain the channel's ability to conduct the ion movement at very high rate and relate it to the detailed molecular structure of the system. Although the precise geometry of ion-transport pathway varies among different channels, there are

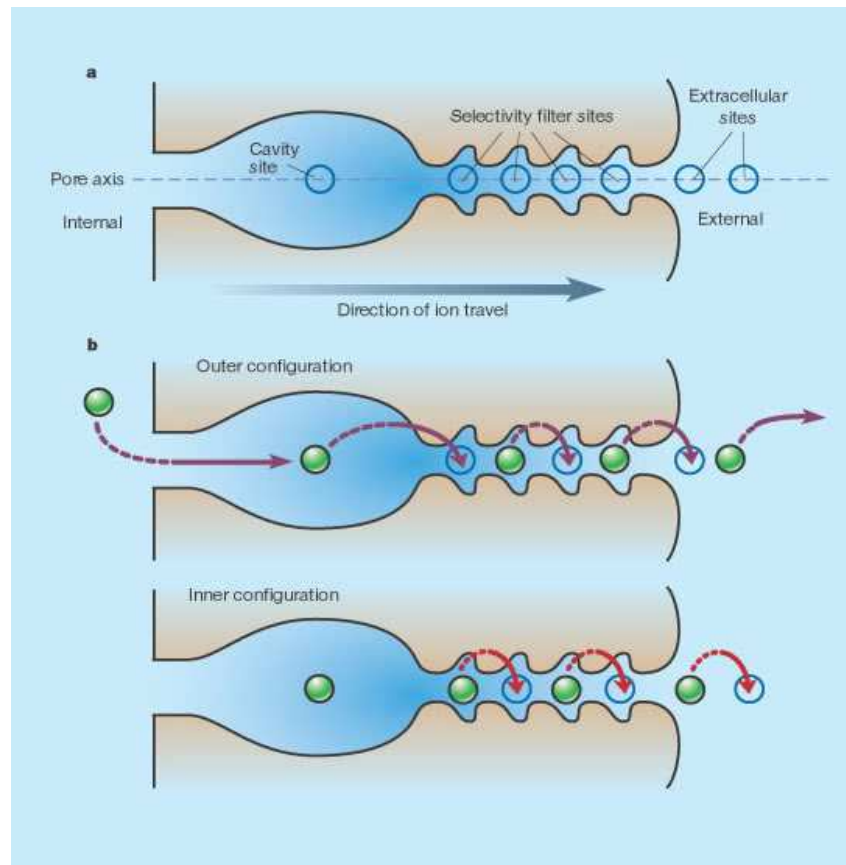


Fig. 4: Permeation process in the KcsA ion channel. (a), There are seven main sites for ions along the pore axis: one in the pore cavity, four in the selectivity filter and two just beyond the external end of the pore. The cavity site is fully occupied, but (as indicated in b) only half of the remaining six are occupied at any one time. (b), The two main ion configurations, known as outer and inner, that are postulated to exist within the pore. Purple arrows indicate ion shifts that are linked directly to concerted ion entry into and exit from the pore. Red arrows represent shifts within the pore without ion entry and exit. As shown here, then, ion passage through the selectivity filter and extracellular sites occurs in bucket-brigade fashion. (From [53]).

two characteristic regions that appear to be a general feature of all biological channels (Fig.4) : a wider cavity that accommodates hydrated ion and short narrow selectivity filter. Those two regions seem to be evolutionary conserved, at least in specific channel type (e.g. potassium channels). The mechanism of ion permeation through the channel is generally determined by interactions of permeating ion with the channel wall and ion-ion interaction within the channel pore. Additionally, the interactions are modified with the physical determinants of water molecules within the narrow channel structure, that can be very much different from the bulk water outside the protein structure.

Selectivity. For every experimentally identified ion channel, there is a well defined ion selection sequence, according to which channels are usually named as potassium, sodium, chlorine or calcium channels. It appears that for the monovalent-selective channels the main selection criterion is the size of the ion, whereas for the calcium channel it is the magnitude of ion charge (calcium channel highly discriminates Na^+ over Ca^{2+} , although the radii of Na^+ and Ca^{2+} do not differ much - 0,95 vs. 0,99 Å , respectively). Thus, there are dominantly two different se-

lection mechanisms at play; the one operates on the basis of ion size, and the other on the basis of ion charge. The deciding factor on selectivity in channels is the free energy of permeation, namely the variation in free energy of the system as different ion species pass through the channel. In the simple case one can observe the differences in potential energies at various points along the pore, but for a more quantitative description, a free-energy calculations are needed.

Gating. Ion channels regulate the selective transfer of ions across the membrane in response to different types of stimuli, as e.g. changes of pH, transmembrane potential, mechanical stress or ligand binding. A channel can generally assume two stable conformations, the open and the closed one. The structural part of the channel responsible for the gating controls the accessibility of ions to a centrally located water-filled pore. The opening and closing of the gate is accompanied by conformational changes in the protein during gating. The structural and dynamical details of the gating mechanism are the least known properties of ion channels, mostly because of the fact that an opened state of the channel is a transient one, thus not easily fixed to be isolated by crystallization. Consequently, for the majority of known structures no direct comparison of X-ray structures is available and one has to use other experimental techniques that reveal the structural determinants of the gating mechanism.

AChR

The nicotinic acetylcholine receptor (nAChR) is one of most intensively studied ligand gated ion channels, and thus provides a paradigm for the molecular mechanism of fast synaptic neurotransmission. Depolarization of the presynaptic membrane causes release of acetylcholine into the synaptic cleft. Acetylcholine diffuses to the postsynaptic membrane where it binds to the extracellular domains of the nAChR. Details of the structure of the nAChR have been unveiled recently [54]. The nAChR is pentameric, the five subunits arranged around a central pore (Fig.5b,c). The transmembrane pore is lined by the second transmembrane helix (M2) of each subunit. Binding of acetylcholine to the extracellular domains is thought to initiate a wave of conformational change that is propagated through the protein into the transmembrane domain and the inner (M2) helices. This results in a 'twist to open' [54] mechanism of channel gating whereby the central pore is opened to the permeation of cations (Fig.5a). The ACh-induced rotations in the α -subunits are transmitted to the gate - a hydrophobic barrier to ion permeation - through the M2 helices. The rotations destabilize the gate, causing the helices to adopt an alternative configuration which is permeable to the ions. The helices move freely during gating because they are mainly separated from the outer protein wall and connected to it by flexible loops, containing glycine residues (G). S-S is the disulphide-bridge pivot in the ligand-binding domain, which is anchored to the fixed outer shell of the pore. Binding of ACh opens the channel by initiating rotational movements (arrows) of the inner β -sheets of the α subunits in the ligand-binding domain. The rotations destabilize the gate, causing the helices to adopt an alternative configuration which is permeable to the ions. These movements are communicated to the inner (M2) helices lining the pore and break apart the gate - a hydrophobic girdle in the middle of the membrane - so that ions can flow through. The helices move freely during gating because they are mainly separated from the outer protein wall and connected to it by flexible loops, containing glycine residues (G). S-S is the disulphide-bridge pivot in the ligand-binding domain, which is anchored to the fixed outer shell of the pore.

Potassium Ion Channel KcsA

A major breakthrough in our understanding of ion channels at the atomic level had taken place after the recent successful structural determination of the bacterial potassium channel KcsA

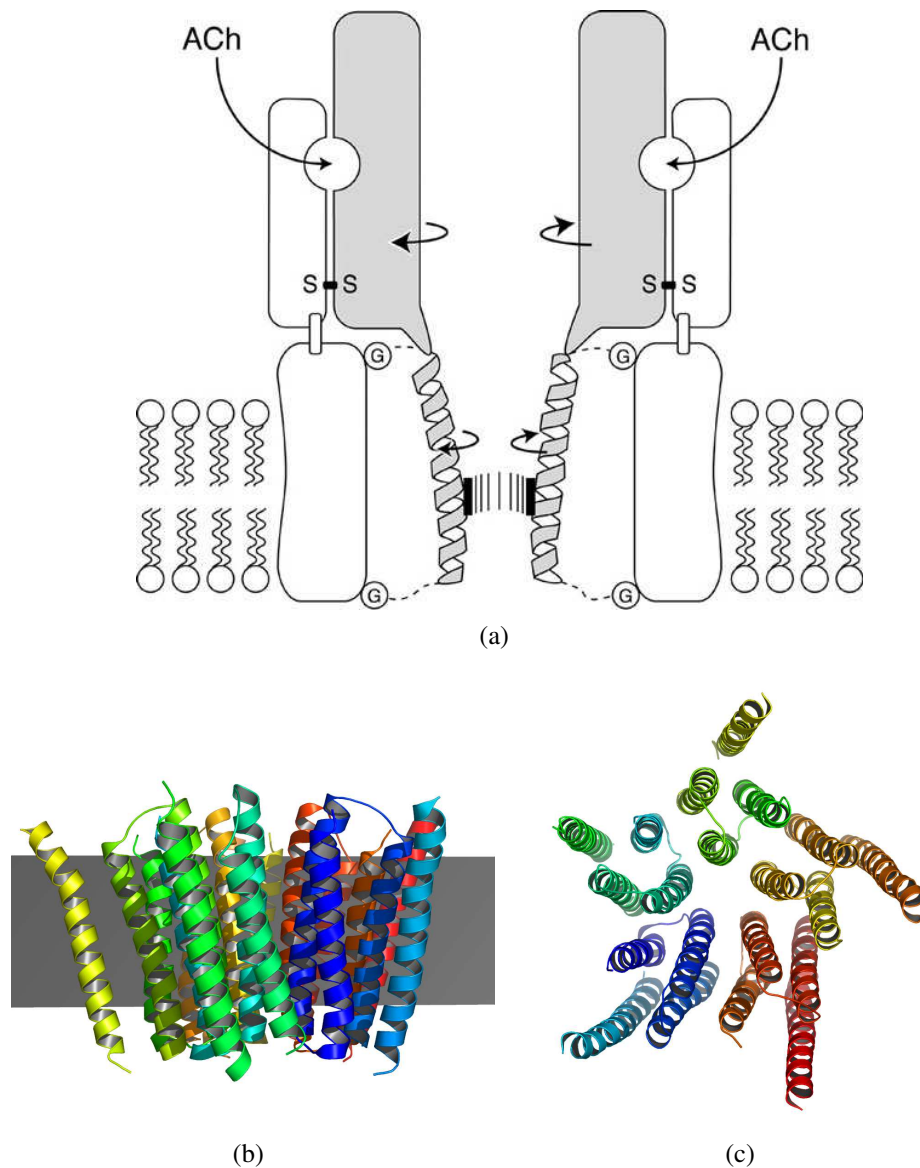


Fig. 5: (a) Proposed model for the gating mechanism (for details see text). The relevant moving parts are shaded. The height of the membrane-spanning pore is about 40 Å, the β -sheet structure about 60 Å. (From [54]) (b) Side view and (c) top view of the pore, as seen from the synaptic cleft, with subunits shown in different colors (PDB code 1OED).

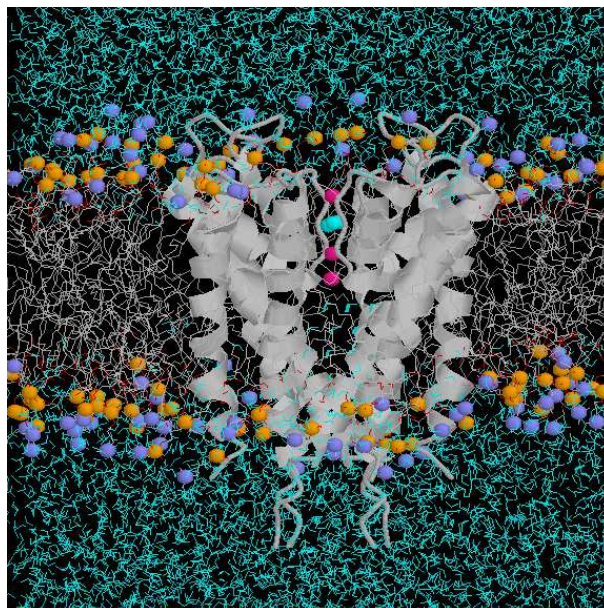


Fig. 6: Structure of the KcsA potassium channel embedded in a fully hydrated lipid bilayer membrane.

from *Streptomyces lividans* by MacKinnon and coworkers [46–49]. Several MD simulations [55–60] have been used to study the structure and dynamics of this ion channel. The structure of the KcsA channel is depicted in Fig.6.

Structure. MD simulations [57,59] have shown that the simulated atomic structure remained stable and very close to the original structure. The r.m.s. deviation of the C_{α} atoms between the crystal structure and the simulated structure was about 3.7 Å, which is comparable to the crystal structure of 3.2 Å resolution [46] and 2.0 Å resolution [48]. MD simulations [57,59,61] indicate that one or at most two ions may occupy the filter simultaneously. The MD simulations predict an average number of water molecules within the cavity of about 21.

Permeation. Our current understanding of ion transport through biological potassium ion channels is based on the concept of the multi-ion transport mechanism: permeating ions line in a queue in the narrow channel pore and move in a single file through the filter (Fig.4). The multi-ion concept had been accepted for many decades, its molecular mechanism, however, remained still elusive. The mechanisms underlying transport of ions across the potassium channel have been examined using electrostatic calculations, three-dimensional Brownian dynamics simulations [62] and MD simulations [60]. More insights have been obtained recently using molecular dynamics simulations [61]. MD simulations permitted to monitor the collective motion of ions and water molecules through the narrow selectivity filter. The simulations reveal that the high conductivity is based on the cooperative diffusion of ions and water molecules mediated by the charged flexible carbonyl groups lining the selectivity filter. A detailed analysis has shown the following microscopic mechanism of ion permeation to be valid. At rest, the queue of ions and intercalated water molecules, e.g., K1-W1-K2-W2, are residing at the minima of the periodic pore potential made by the pore lining carbonyl groups, C-O. The pore exiting ion K1 reorients and attracts the neighboring water molecule W1, which thereby induces a local transformation of the neighboring pore potential where the ion K2 is located. This neighboring pore potential is an asymmetric double well potential, bistable, and shelters the K2 in the lower one of its

two minima. The W1-induced transformation switches the lower minimum from one to the other well, towards the position of W1. Hence, K2 moves towards this new minimum, thereby following the movements of K1 and W1.

Selectivity. Another focus of MD simulations of the KcsA potassium channel has been the selectivity filter and the permeation process from the cavity to the filter. The question of selectivity against Na^+ ions has been addressed in several studies through free-energy perturbation calculations, where a K^+ ion in one of the binding sites is alchemically transformed into a Na^+ ion. the calculated free-energy barrier range from 11 kT to 8 kT [63], and 5 kT [64] which are in rough agreement with the experimental value of 9 kT extracted from the K^+/Na^+ selectivity ratio of about 10^4 . The selectivity is based on the differences in the dehydration energy between K^+ and Na^+ ions.

Gating. The *Streptomyces lividans* potassium channel (KcsA) is pH regulated [65]. A gating mechanism was proposed by Perozo and coworkers [66–68] by using site-directed spin-labeling methods and electron paramagnetic resonance spectroscopy. Results from these experiments indicate that the channel undergoes a “twisted” motion where each of the four TM2 helices tilts away from the permeation pathway, towards the membrane plane, and rotates about its helical axis, supporting a scissoring-type motion with a pivot point near residues 107-108. These movements result in a large increase of the diameter of the intracellular mouth up to the central water-filled cavity. Although the possible collective motion of the helices can be constructed, mainly based on steric considerations, the origin of the pH-mediated driving force is still unclear. Unfortunately the gating mechanism cannot be studied by standard MD simulation because the time scale of gating is in the order of at least microseconds. The simulation of channel gating is therefore still one of the great challenges in biophysics.

4.2 Membrane Transporters

The exchange of substances across cellular membranes may be accomplished by diffusion, facilitated diffusion, or active transport. Active transport is necessary to accumulate a substance against a concentration gradient. Thermodynamics require some kind of energy to perform this, so there has to be another downhill gradient that may be dissipated or some other form of chemical energy. The transport uses energy directly: light or chemical energy is converted to electrochemical energy as electrochemical potential of the substances to be transported. This category comprises photosynthetic electron transport, light driven ion pumps, redoxenergy dependent respiratory chains, transport ATPases and sodium pumps utilizing decarboxylation energy. The structures of few transport systems are known at atomic resolution. Among these are rhodopsins, the proton transporters bacteriorhodopsin [69] and V-ATPase, the ion transporter Ca-ATPase, and the protein transporters as mitochondrial and endoplasmatic pores, and the nuclear pore complex.

V-ATPases

Structure and Function. Intracellular compartments exhibit a significant difference between their luminal pH and that of the bulk cytoplasm. This pH difference is maintained mostly by the vacuolar H^+ -ATPases (or V-ATPases), which is a proton pump. V-ATPases are multisubunit complexes (Fig.7) composed of a peripheral domain (V_1) responsible for ATP hydrolysis and an integral domain (V_0) responsible for proton translocation. The V-ATPases are thought to operate

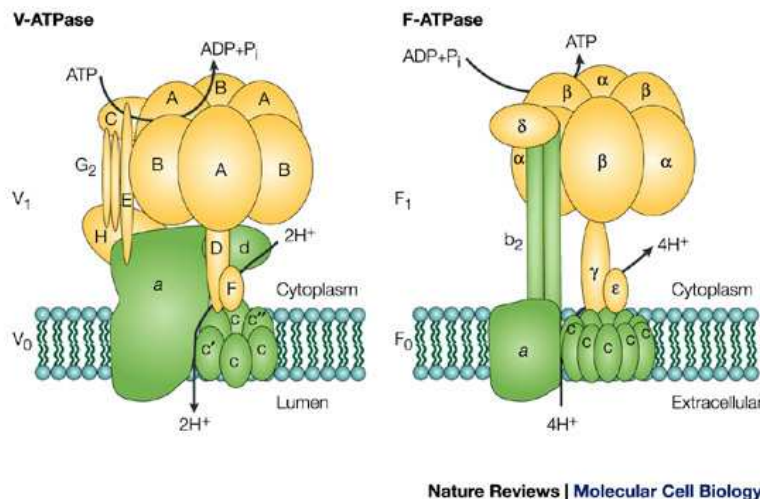


Fig. 7: Structural model of the V-ATPase and comparison with the F-ATPase. The V-ATPases are composed of a peripheral V_1 domain responsible for ATP hydrolysis and an integral V_0 domain responsible for proton translocation. The corresponding domains for the F-ATPases are F_1 and F_0 . The nucleotide binding sites are located on the A and B subunits of V_1 , with the catalytic sites located primarily on the A subunits. Proton translocation is postulated to occur at the interface of the a subunit and the ring of proteolipid subunits (c, c' and c''). The V_1 and V_0 domains are connected by both a central stalk (composed of the D and F subunits) and a peripheral stalk (composed of the C, E, G and H subunits). These stalks play a crucial role in the assumed rotary mechanism of ATP-driven proton transport (From [70]).

by a rotary mechanism in which ATP hydrolysis in V_1 drives rotation of a ring of proteolipid subunits in V_0 . The peripheral V_1 domain contains eight different subunits (subunits A-H). The integral V_0 domain, responsible for proton translocation, is composed of five subunits (a, b, c, c', c''). A model for the arrangement of subunits in the V-ATPase complex is shown in Fig.7.

Theory. A mechanochemical model of V-ATPase proton pump has been proposed by Oster, Grabe and Wang [71]. They used a mathematical formulation of the model where the motion of the rotor, in terms of its rotation angle $\theta(t)$, was computed from a force balance equating the viscous drag on the rotor to the torques that act on the rotor and the Brownian force modeling the rotor's thermal fluctuations by a Langevin-type equation

$$\zeta \frac{d\theta}{dt} = \tau_Q(\theta, s) + \tau_\psi(\theta, s) + \tau_\epsilon(\theta, s) - \tau_D(\theta) + \tau_B(t).$$

The various terms correspond to the rotator-stator charge interaction, the membrane potential, the dielectric barrier, the driving torque from V_1 , and the Brownian torque, respectively. The chemical states of the rotor are described by the binary variable s_i for full or empty, and determined by a Markov equation $ds/dt = K(\theta)s$, where $K(\theta)$ is the transition matrix. Using appropriate parameters, the model calculations reproduced a variety of experimental measurements of performance of the V-ATPase proton pump .

4.3 Motor Proteins

Many essential functions of biological cells are performed by nanoscale motor proteins . They use the cell's chemical energy repositories of phosphate bonds in nucleotides, generally ATP

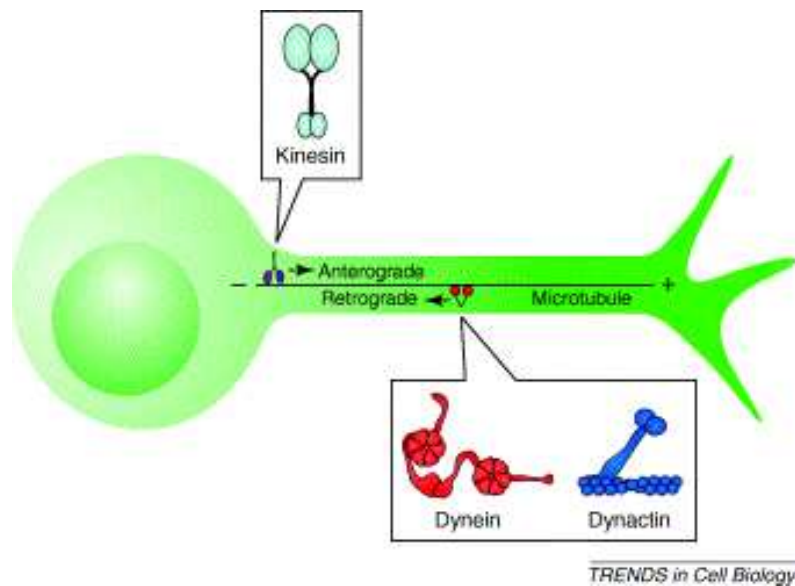


Fig. 8: Microtubule motors (kinesin, dynein, dynactin) drive axonal transport in neurons. (From [73].)

or GTP. These biomolecular motors make use of the ATP hydrolysis as the free energy source. The motor proteins have a wide range of functions, including organelle transport, muscle contraction, and translocation along DNA/RNA. They play an important role in cell division and cellular motion. The malfunction of certain motor proteins can be a source of disease, such as structural myosin mutations in the head and neck region of the molecule. An understanding of the mechanisms of these motor proteins may provide not only a guide for therapy, but may also for developing prototypes of hybrid dynamic nano-devices that operate on micro- or nano-fabricated structures with outstanding efficiency and diversity as biological motor proteins.

The most widely used mechanism for intracellular transport involves molecular motor proteins that carry cargo directionally along a cytoskeletal track :

myosins along actin filaments, anterograde,

kinesins along microtubules, anterograde,

dyneins along microtubules. retrograde.

Therefore, they are also called ‘linear molecular motors’ or ‘track motors’. A recent book by J. Howard [72] reviewed in detail many aspects of the biophysics of linear molecular motors. The structures of kinesins, dyneins and myosins have so-called ‘motor domains’ that move along the tracks, microtubules or actin filaments in a specific direction. Kinesin and dynein proteins are microtubule-dependent motors that slide along microtubules, long hollow cylinders of 25 nm in diameter built by tubulin polymerisation, whereas myosin proteins are actin-dependent motors that slide along thin actin filaments or microfilaments of 6 nm in diameter. Kinesin uses the energy of ATP hydrolysis to move along microtubules. Most kinesins move to the plus end of the microtubules, whereas dynein proteins move to the minus end (Fig.8). Most members of the myosin proteins move to the plus end of the actin filaments. Some of the plus-end-directed kinesins such as transport cargoes to the dendrites, but most of them transport cargoes to the axons. These motors use the binding energy of the fuel molecule ATP to the enzyme to produce mechanical force, and they use the hydrolysis reaction to weaken the binding between the enzyme and products so that they can be released and the cycle can repeat. Fig.8 shows how

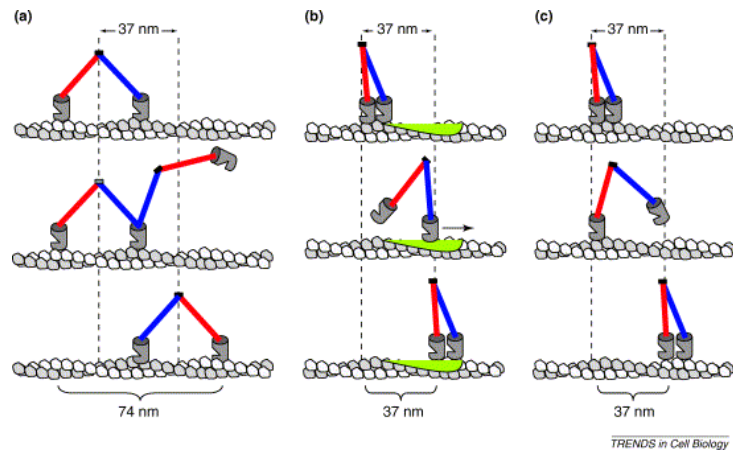


Fig. 9: Processivity of myosin-V on actin filament : motility models. (From [81])

microtubule motors drive axonal transport in neurons. Neurons rely on anterograde transport, powered by kinesin to supply newly synthesized material to the axon and presynaptic terminal. Retrograde axonal transport, driven by cytoplasmic dynein and dynactin, returns material targeted for degradation back to the cell body.

The pioneering works of Oster [74] and Leibler [75] have provided one of the first mathematical formulations of phenomenological models of motor proteins. The first paper contains a model for single-motor proteins (myosin, dynein, kinesin) that is powered either by thermal fluctuations or by conformational change. The second paper presents a general phenomenological theory for chemical to mechanical work transduction by motor proteins based on the classical ‘tight-coupling’ mechanism included in a minimal stochastic model. Many theoretical studies are summarized in recent reviews [76, 77], and we refer the reader to these excellent reviews for earlier approaches.

MYOSIN V

Myosin-V is a motor protein responsible for organelle and vesicle transport in cells. Several mechanisms [78, 79] and theoretical models [80] have been proposed to explain the observed data. Single-molecule experiments have shown that it is an efficient processive motor where ATP hydrolysis energy fuels the translational movement of myosin-V toward the plus end of actin. Single-molecule mechanical assays have shown that myosin-V moves processively along actin filaments by taking large, ~ 37 nm steps. The large size of these steps corresponds to the linear repeat of binding sites that appear along the helical actin filament. Electron microscopy along with kinetic analyses (e.g. [82, 83]) indicates a hand-over-hand fashion [model (a) Fig.9], walking along the filament, with the two motor domains taking turns in the lead. Much of the present experimental data for myosin-V can be well described by a two-state chemical kinetic model [80] with three load-dependent rates. The analysis predicted the variation of the mean velocity and of the randomness with ATP concentration under both resisting and assisting loads.

DNA POLYMERASES

DNA polymerases are responsible for replication of normal and damaged DNA, and faithful DNA replication is crucial for genomic stability. The actual motor mechanism, which is the translocation of DNA polymerase along the DNA, is not well understood. General theoretical considerations about the mechanism of DNA polymerization are based on the Brownian ratchet

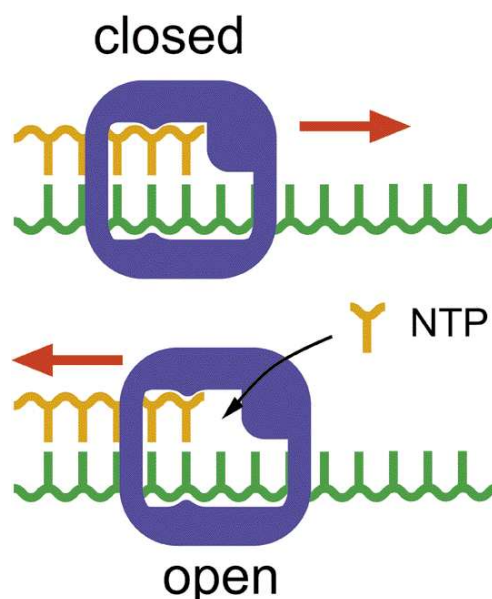


Fig. 10: *Brownian ratchet model for DNA polymerase. (From [77])*

concept [77]. In a Brownian ratchet, the role of the chemistry is to select forward fluctuations (or prevent backward fluctuations) of the load, rather than to apply a mechanical force directly to the load. That is, the load force is driven by its own Brownian fluctuations, and the chemistry provides the energy to rectify the diffusive motion of the load. Though this is a slightly more subtle mechanism than the power stroke, even very simple mechanisms can act as Brownian ratchets. For example, consider the simple model for a DNA polymerase shown in Fig.10. A DNA polymerase enzyme is bound at the junction between double-stranded DNA and single-stranded DNA. It can slide rapidly between a "closed" state and an "open" state one step forward by opening a space for a new nucleotide (NTP), but no farther. While in the open state, a new nucleotide can bind and be incorporated into the growing strand, thus locking the polymerase one step ahead. Both open and closed states have equal free energy, so, though it can step back and forth under the influence of thermal fluctuations, neither is preferred statistically, and no net mechanical forces push the molecule either forward or backward. Net forward motion occurs because binding of a new nucleotide in the open state prevents the backward step. This physical model can be described by a two-step kinetic scheme, where the single chemical step includes both binding and incorporation of the nucleotide into the chain. In this example, the motor moves forward because the chemical step is irreversible, i.e., its free energy of reaction is large and negative. This example clearly illustrates that the chemical energy is expended to preferentially select forward steps (or prevent backward steps) and hence to favor forward motion, rather than doing mechanical work on the motor directly. A full description of this molecular motor can be given either in a continuum model by the Smoluchowski equation, or in a discrete model by kinetic equations, or in a "mixed model" in which the chemistry is discrete but the mechanics are continuous [77].

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