1 Physicochemical Kinetics and Transport at the Biointerface: Setting the Stage

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Life has developed in media with very diverse chemical compositions and with a variety of physical conditions, including temperature, pressure and their gradients. Evolution actually implies an optimisation in the functioning of organisms in response to these physical and chemical conditions in which they live. It follows that a change in conditions will give rise to a change in the properties of the organism, and this is known in biology as *adaptation*. The chemical conditions relevant to survival, evolution and adaptation comprise not only the composition and the chemical dynamics of the medium in which the organism is living, but also the *availability* of the various chemical species. Therefore the distribution and mobilities of inorganic and organic materials in abiotic and biotic media are of paramount importance in understanding their fate and effects in environmental systems. The present book is concerned with the coupling between environmental media and biota, and focuses on the physicochemical features of processes at their interphases.[1]

Every living cell, whether it be a unicellular organism on its own or a part of a multicellular organisation, is encircled by a biological membrane. In this context, the terms 'cell membrane', 'plasma membrane', and 'cytoplasmic membrane' are used synonymously. Generally, the interphase between an organism and its environment encompasses the elements outlined in Figure 1. The scheme shows that the cell membrane, with its hydrophobic lipid core, has the most

[1] Depending on the context, we sometimes prefer the term 'interphase' over 'interface' because the latter refers to an infinitely sharp dividing plane between two phases. Organisms generally form boundary layers, e.g. the cell wall, that are characterised by a gradual transition from the biological phase to the medium phase, and if we discuss the volume properties of such layers the term 'interphase' is more appropriate.

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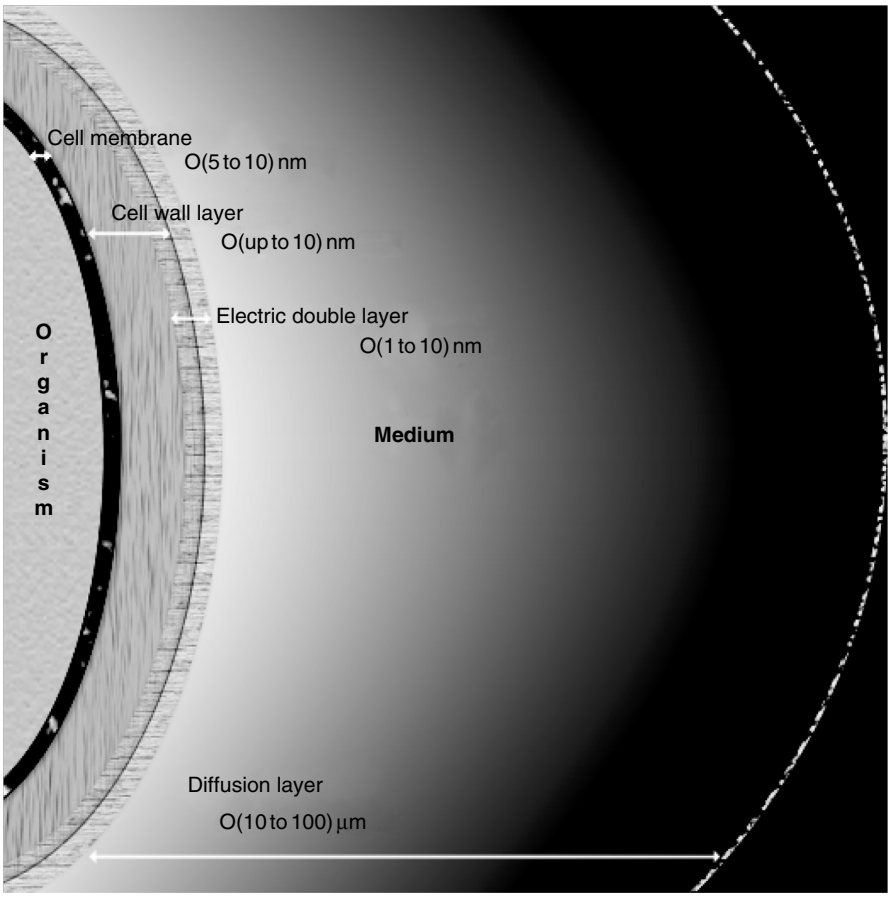

Figure 1. Schematic outline of the typical dimensions of the various physically relevant layers at the organism/medium interphase: cell membrane, cell wall layer, electric double layer, diffusive depletion layer

prominent function in separating the hydrophilic aqueous medium from the interior of the cell. The limited and selective permeabilities of the cell membrane towards components of the medium, be they nutrients or toxic species, play a key role in the transport of material from the medium towards the surface of the organism.

The lipid bilayer has a very low water content and its core behaves quite hydrophobically, while the cell wall is rather hydrophilic, containing some 80% of water. Physicochemically, the cell wall is particularly relevant because of its high ion binding capacity, and the ensuing impact on the biointerphasial electric double layer. The presence of such an electric double layer ensures that the cell

wall possesses Donnan partition characteristics, leaving only a limited part of the interphasial potential decay in the diffuse double layer of the adjacent medium.

Mass transfer phenomena usually are very effective on distance scales much larger than the dimensions of the cell wall and the double layer dimensions. Thicknesses of steady-state diffusion layers[1] in mildly stirred systems are of the order of 10^{-5} m. Thus, one may generally adopt a picture where the local interphasial properties define boundary conditions while the actual mass transfer processes take place on a much larger spatial scale.

The availability of chemical species to organisms is defined by a number of basic features, including:

- their chemical reactivity, as derived from equilibrium distributions of species and their rates of interconversion;
- the supply (flux) of these chemical species to the relevant sites at the surface of the organism, as governed by their mass transport properties and the concentration gradients that arise at the interphase as a consequence of the interplay between chemical reactivity and biological affinity; and
- the internalisation of the chemical species, governed by an internalisation rate constant, k_{int}, usually accompanied or followed by some bioconversion process.

The actual processes of uptake of chemical species by an organism typically encompass transport in the medium, adsorption at extracellular cell wall components, and internalisation by transfer through the cell membrane. Each of these steps constitutes a broad spectrum of physicochemical aspects, including chemical interactions between relevant components, electrostatic interactions, elementary chemical kinetics (in this volume, as pertains to the interface), diffusion limitations of mass transfer processes, etc.

Life on Earth in all its diversity could never have evolved without the existence of lipids that are able to spontaneously arrange in aqueous solutions into structures such as micelles or bilayers. Although the composition of biological membranes varies markedly with their various functions, and with the type of organism and environmental conditions, there is a common structural organisation involving lipid, protein, and carbohydrate components. With only a few exceptions, lipids are arranged as bilayers and constitute the basic characteristic architecture for a variety of biomembranes found in different living organisms. The average thickness of biomembranes is approximately 7 nm. The majority of lipids in the plasma membrane of bacteria (prokaryotes)

[1] Such layers are frequently denoted as 'unstirred' layers. The term 'unstirred' however, is physically incorrect [1], since velocity profiles in liquid media are continuous functions which only approach zero at the actual interface. In gel layers the liquid velocity is generally low, but this is due to their high viscosities.

and eukaryotes are comprised of acyl chains (e.g. palmitic acid, stearic acid, oleic acid), which are ester-linked to glycerol. However, many other more complex lipids that contain additional elements like phosphorus, nitrogen or sulfur, may also be found in biomembranes. In addition, hydrophilic components such as small sugars, choline, serine, or ethanolamine are commonly found. Phospholipids containing a phosphate group are ideal amphipathic components, and represent the largest group of membrane lipids. Sterols like cholesterol are almost exclusively found in eukaryotic membranes, where they can make up to 25% of the total lipids. Archaebacteria can exist in the most extreme conditions, and their membrane composition differs from those of the bacteria and eukaryotes. Some unusual components like hopanoids have been found in this group of organisms. Hopanoids are pentacyclic triterpenoids, biosynthetically derived from the linear molecule squalen, which is formed by joining six isopentenyl units. It is assumed that hopanoids may play a role similar to that of sterols in eukaryotic cells. Another common feature of archaeal membranes are acyl chains derived from repeating units of isoprene (e.g. phytanol) which are ether-linked to glycerol or nonitol. The membranes of hyperthermophilic Archaea living at high temperatures are composed of glycerol di-ethers and glycerol tetra-ethers. Lipids containing biphytanyl chains can form monolayers (resembling somewhat the usually found bilayers) with a hydrophobic milieu inside and hydrophilic surfaces outside. They are very stable under extremely high temperatures.

Proteins represent another major group of membrane components. They play structural roles and/or are involved in many cellular processes, which are strictly coupled to membranes. Proteins can be either entirely embedded within the bilayer, or they might be firmly anchored (e.g. by a hydrophobic transmembrane segment composed of hydrophobic amino acid side-chains or as lipoprotein), or they can be just associated with the surface.

Carbohydrates related to membranes can be found as lipopolysaccharides or as parts of glycoproteins. Sugars are often characteristic determinants of cell surfaces (see below). The great majority of carbohydrates are found in the outer leaflet of a membrane, resulting in an asymmetrical structure. This is especially true for many plasma membranes and the outer membrane of Gram-negative bacterial cells (see below).

The membrane is the regulating barrier for exchange of chemical species between the environmental medium and cell interior. It may be practically impermeable to one type of species and highly permeable to another. In the chain of transport steps from the bulk of the medium to the cell interior, the membrane transfer step may thus vary from fully rate-limiting to apparently fast with respect to transport in the medium. The overall rate of this biouptake process is determined by mass transport either in the medium or through the membrane: the actual rate-limiting step will depend on a large variety of factors. Membrane

transfer rates may be influenced by external chemical conditions, such as pH, ionic strength, presence of surfactants, etc., which alter the permeability features of the membrane, as well as by biological factors like conditioning and adaptation, which may regulate the effectiveness and abundance of transporter functions inside the membrane. An intact and largely undisturbed cytoplasmic membrane or plasma membrane representing the innermost layer enclosing a biological cell is absolutely essential for its vitality. Any major impairment or even a small hole would cause unimpeded exchange of ionic species and thus electrical depolarisation of the membrane, resulting in immediate cell death. This effect can also be generated by certain toxins, which assemble into pores in the membrane. Therefore, channels that are simultaneously open to both sides of the cytoplasmic membrane cannot persist in a living cell.

With the help of the atomic force microscopy (AFM) technique, it is possible to obtain three-dimensional images of surface structures at the nanometre scale. Erythrocyte membranes, which are stable during the preparation of an AFM experiment, can be used as a rather basic model with respect to composition and surface structure. This enables a number of details to be visualised, e.g. the deformation of a rhesus monkey erythrocyte membrane caused by an infecting virus (Figure 2). In most cases, however, a given solute approaching the surface of a living cell has to deal with more complex structures, a 'naked' membrane surface being highly unusual. In human or animal cells, various glycopeptides and glycosylated proteins are integrated into the lipid bilayer, while most plant

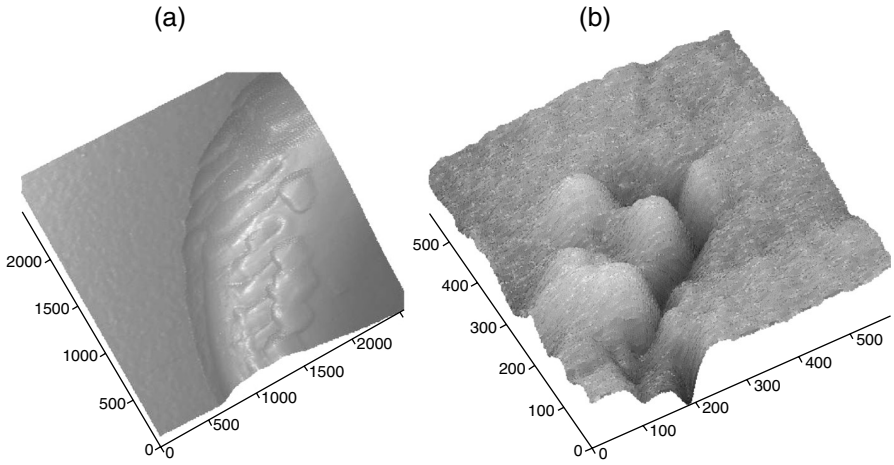

Figure 2. Atomic force microscopy images showing the surface of a rhesus monkey erythrocyte membrane. Damage, such as formation of humps on the peripheral surface and pits in other parts, results from the interaction with virions of the canine parvovirus. (**a**) edge of erythrocyte; (**b**) pits on membrane surface. (Source: http://www.ntmdt.ru/publications/download/211.pdf, Reproduced with permission from Dr Boris N. Zaitser)

cells are surrounded by a cell wall composed of polymers of carbohydrates. Bacteria are usually encircled by a sacculus: this peptidoglycan (or murein) sheet contains glycan chains formed by the alternating sugar derivatives *N*-acetylglucosamine and *N*-acetylmuramic acid, which are cross-linked by small peptides building a network. Gram-positive bacteria are characterised by multiple layers of peptidoglycan with attached teichoic acids (acidic polysaccharides consisting predominantly of glycerophosphate mannitol phosphate or ribitol phosphate). Alternatively, Gram-negative bacteria possess only a single peptidoglycan layer, but additionally have a second membrane, called the 'outer membrane', harbouring various proteins, lipoproteins, and lipopolysaccharides. Moreover, a number of bacterial species produce external capsules or slime layers, while others are capable of building spores that are highly resistant to adverse environmental conditions.

This book focuses on the processes that control the transfer of chemicals between environmental media and living organisms. The major driving forces for transport and chemical conversion are contained in the electrochemical potential, that is, the chemical potential difference plus the electrostatic free-energy change for charged species. Electrical potential differences between the inner and outer boundary of the biological membrane play a crucial role in the various physiological mechanisms. Such potential differences, usually denoted as membrane potentials, derive from differences in permeability of the membrane with respect to ions in the inner and outer media. Common membrane potential expressions, like the Goldman–Hodgkin–Katz equation [2,3] for Na^+, K^+ and Cl^-, are valid under steady-state conditions of zero net charge transport:

$$\sum_i z_i J_i = 0 \tag{1}$$

where i indexes the permeable ionic species, z_i is the charge number of i, and J_i is the flux[1] of i across the membrane. Fluxes of charged species are generally the result of gradients in concentration, potential and pressure, which are collectively represented by the electrochemical thermodynamic potential $\tilde{\mu}$.

Application of elementary conservation laws leads to formulation of a general expression for J_i, which is often denoted as the Nernst–Planck equation:

$$J_i = -D_i \text{ grad } c_i - (z_i/|z_i|)\, c_i\, u_\text{i} \text{ grad } \Psi + c_i \nu \tag{2}$$

[1] For convenience, fluxes from the medium towards the organism will be counted as positive throughout this book. Analysis of mass transport in the medium is usually based on a coordinate system with the origin at the interface and a positive axis going outward, leading to a negative sign in fluxes towards the interface.

Equation (2), where D denotes diffusion coefficient, c concentration, u mobility, Ψ electric potential, and ν flow velocity, explicitly shows the diffusion, conduction and flow terms respectively. Within the context of biological systems, transport represented by this Nernst–Planck equation (2) is often referred to as 'passive' transport. This qualification is intended to make a distinction from situations where apparently transport takes place in the opposite direction, against a gradient of concentration or potential or pressure. Obviously, such 'uphill' or 'active' transport requires special conditions, which in biomembrane transport are created by metabolic chemical reactions such as ATP hydrolysis. The coupling of the ionic transport process with the energy providing chemical reaction must be of an *asymmetrical* nature, in the sense that the production/consumption of ions at the inner side of the membrane is different from that at the outer side. It has been hypothesised that the asymmetry is in the *kinetic features* of the interfacial transfer process, in such a way that, in the apparent steady-state, the ratio between influx and efflux is modified. Under such conditions, which essentially are of a nonequilibrium nature, it is possible to realise net uphill ionic transport, and this is the basis of the biologically well-known ionic pumps. The existence of ionic pumps is not in conflict with fundamental transport laws like the Nernst–Planck equation (2): these pumps are generated by the special geometrical and chemically asymmetrical conditions in a biological membrane. In fact, for a rigorous analysis of the pump situation, the Nernst–Planck conservation equation has to be complemented with a chemical source term with a confined spatial distribution.

Transport across biological membranes is facilitated by their fluid-like nature. The water content varies strongly from the core to the outer boundary; overall it comes to some 25% by mass. The classical Singer–Nicholson fluid mosaic model represents the biomembrane as a two-dimensional sea of the lipid bilayer, in which proteins and other constituents are floating around. Indeed, most lipid membranes are fluid at physiological temperature, and consequently the lateral mobility of the lipids and proteins is relatively high, whereas the transversal movements (including the flip-flop exchange of lipids between the inner and outer sides) are strongly limited. This feature explains the maintenance of the asymmetry of the membrane with respect to composition and orientation of the ion transporter proteins. As outlined above, this chemical asymmetry is essential for the basic functioning of the biomembrane. Below a certain temperature, the fluid bilayer turns into the crystalline–gel state, in which the lateral mobility of the constituents is greatly diminished.

In the fluid state, the lateral diffusion coefficient of lipids in the bilayer structure is $O(10^{-13})\,m^2\,s^{-1}$ (the symbol 'O' is used to indicate order of magnitude). Interestingly, it has been shown that the diffusion coefficients of phospholipids may differ greatly from the inner to the outer leaflet of the biomembrane layer [4,5]. Again, this is related to the differences in chemical

composition. Lateral transport of lyophobic species like water and ions in the core of the bilayer is not very relevant, because of their extremely low local concentrations. Mobilities of ions in the interphasial region, even inside the stagnant water layer at the actual interface between the aqueous phase and the lipid bilayer, are on the level of that in the bulk solution [6].

As noted above, biouptake involves a series of elementary processes that take place in the external medium, in the interphasial region, and within the cell itself. One of the most important characteristics of the medium is the chemical *speciation* of the bioactive element or compound under consideration. Speciation not only includes complexation of metal ions by various types of ligands, but also the distribution over different oxidation states, e.g. Fe(II) and Fe(III), and protonation/deprotonation of organic and inorganic acids of intermediate strength. The relationship between speciation and the direct or indirect *bioavailability*[1] of certain species has received a lot of recent attention.

Organisms are able to take advantage of a wide range of nutrients, ranging from trace elements to biopolymers such as proteins, DNA, RNA, starch, lignin, etc. Although they are often present in relatively large amounts, these compounds are not always accessible, as illustrated by the following examples:

(1) iron, which is an essential nutrient for most living bacteria (lactobacilli being the only notable exception), is the fourth most abundant metal on Earth. However, iron is not readily bioavailable under 'normal' physiological conditions. In the environment it is mainly found as a component of insoluble hydroxides; while in biological systems it is chelated by high-affinity iron-binding proteins (e.g. transferrins, lactoferrins, ferritins) or found as a component of erythrocytes (haem, haemoglobin, haemopexin). As a consequence, organisms have evolved a number of different sequestering strategies for this metal. Under anaerobic conditions, ferrous iron can be transported without the involvement of any chelators. Likewise, at pH 3, ferric iron is soluble enough to support growth of acid-tolerant bacteria. At higher pH values, however, iron is mostly found in insoluble compounds. Therefore, a great variety of low-molar-mass iron ligands, so-called siderophores, which bind Fe^{3+} with very high affinity, are produced by many bacterial species, certain fungi, and some plant species. These chelators are released in their iron-free forms and subsequently transported back into the organism as ferric-siderophore-complexes. Furthermore, a

[1] The notion 'bioavailability' is used with different meanings. Environmental chemists understand it in terms of the supplying potential of the medium, whereas (micro)biologists relate it to the assimilation properties of the organism. In the case of metal uptake, for example, a certain complex may be fully labile and thus potentially contribute to the supply of free metal ions. In contact with an organism with a modest affinity towards the metal in question the uptake requirements may be so small that such labile complexes are completely unimportant and their lability irrelevant. In microbiological jargon this complex would be 'not bioavailable', whereas a chemist would say that this complex is fully available to the organism.

number of organisms are able to use haem-bound iron from haemoglobin and similar molecules. Some bacterial species can acquire iron that is released by an as-yet unknown mechanism from transferrins or lactoferrins, whereas vertebrates take up the whole iron–protein complex. All these processes involve specific uptake systems in the cell envelope and in the cytoplasmic membrane.

(2) although many biopolymers represent an excellent source of nutrients, they are often too large to be transported into a biological cell. A number of species have developed ways to overcome this problem by the secretion of enzymes, which are able to breakdown polymers into their constituents. Many organisms originating from all kingdoms of life are known to use this strategy. So-called exo-enzymes, which are released from the producing cell, can be classified according to their functions (e.g. proteases, lipases, nucleases). Although in some cases these enzymes only carry out a partial degradation, oligomers (e.g. peptides) up to a certain size become 'bioavailable' and can then be transported into the cell.

In particular, the kinetics of dissociation reactions as preceding steps in the biouptake of organics and metals from complex media have been extensively studied. It is likely that the gap between the concentration of labile species, as measured by a certain dynamic analytical technique [7], and the effective bioavailability of that species will soon be bridged. The role of the adsorption of bioactive species in the cell wall region becomes important as soon as a mechanistic interpretation of biouptake fluxes beyond their mere values in the ongoing steady-state is sought. Back-extrapolation of fluxes to zero time, or even better, analysis of the initial transient behaviour of the flux, will provide more comprehensive information on the molecular details of the internalisation kinetics. Such means will enable distinction between receptor sites (physiologically active) and mere adsorption sites (physiologically inactive), metal ion buffering action of the adsorption sites in the cell wall region, and true non-conditional rate constants of the actual membrane transfer steps.

Comprehensive models for the overall biouptake process range from simplifying schemes like the free-ion activity model (FIAM) [8] and the biotic ligand model (BLM) [9] to more differentiated approaches at the level of the Best equation (i.e. Michaelis–Menten control of the uptake and mass transport limitations in the medium) coupled with homogeneous chemical kinetics of formation of the bioactive species in the medium [10–12]. Clearly, the local speciation in the biological interphase may be very different from that in the bulk phase, and this may have a great impact on the nature and rate of bioaccumulation processes. Thus, with the ionic composition of the medium generally being very different from that inside the organism, ion trapping mechanisms may be essential in facilitating efficient transport across the cell membrane.

In addition to their function as a permeability barrier to the extracellular environment, membranes also fulfil important tasks inside most eukaryotic cells and in some bacteria. One crucial role is the separation of different cell compartments. A few examples of intracellular membranes may illustrate the large variety of membrane functions:

- a special type of membrane represents the so-called 'tonoplast' that surrounds the vacuoles characteristic of many plant cells. Vacuoles, which can differ in size, help to maintain the osmotic pressure of the cell, and are used as temporary stores for reserve materials or final storage compartments for waste products of the cell metabolism. The central vacuole of a fully differentiated cell can reach an extensive size, thus constituting the major part of the cell's volume.
- the nuclear envelope consists of an outer and an inner membrane surrounding the nucleus, which harbours most of the genetic information of the eukaryotic cell. The nucleus is the location of, for example, replication, transcription, and RNA processing, and the enzymes involved in these vital functions have to be imported from the cytosol.
- an extensive intracellular membrane system, the so-called endoplasmatic reticulum (ER) is directly connected to the nuclear envelope. A significant portion of protein synthesis is associated with the ER.
- stacks of membranous cistern-like structures (dictyosomes) as well as derived small vesicles and tubules (Golgi vesicles) form the Golgi apparatus. Dictyosomes and Golgi vesicles are involved in intracellular transport and secretion of macromolecules. Exocytosis describes a process in which such vesicles undergo a fusion with the plasma membrane and consequently release enclosed substances into the external medium. Likewise, this membrane flow can occur as a reverse process: endocytosis. In this case invagination of membrane areas leads to intracellular vesicles containing substances from the external medium. This process of 'budding' can also occur in the opposite direction, thus delivering cellular components (or membrane-enclosed phage particles) to the external medium.
- membranes are essential elements of organelles that are exclusively found in plants – the plastids. Among them, the chloroplasts, typical of green plants and algae, display a complex structure. Surrounded by an envelope composed of outer and inner membrane, a complicated system, the thylakoid membranes (see Figure 3), harbours all elements essential for photosynthesis.
- a special compartment, also consisting of an outer and inner membrane, is realised in mitochondria. These organelles contain all components for generating energy in the form of adenosine triphosphate (ATP) via oxidative phosphorylation.

The examples mentioned above exclusively apply to eukaryotic cells. In prokaryotic cells, intracellular membranes are the exception. However,

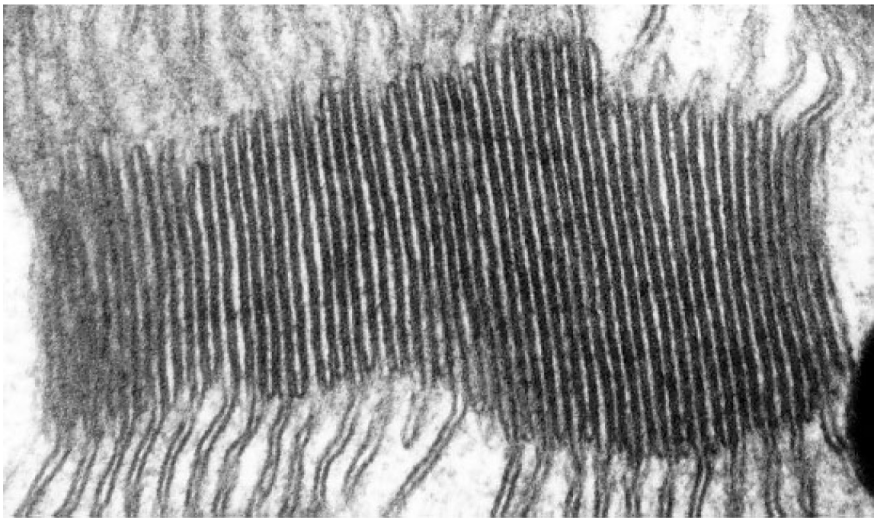

Figure 3. Example of intracellular membrane organisation: a transmission electron microscopy (TEM) image of a section through the thylakoid stack from a chloroplast. (Source: http://www.ru.ac.za/administrative/emu/gr10p6.htm, Reproduced with permission from Dr. R. Cross)

exceptions are known in a few groups of bacteria where complex intracytoplasmic membrane systems result from the invagination of the plasma membrane. Vesicles, tubuli and thylakoid-like structures are reported. Some of them are present in certain phototrophic bacteria. Extensive intracytoplasmic membrane systems are also found in nonphototrophic nitrifying methane-utilising bacteria.

Transport processes across membranes can be divided into several categories:

- transport of signals in the form of a signal transduction cascade can be achieved by a series of conformational changes in the components involved, or by consecutive modification events (e.g. phosphorylation–dephosphorylation, methylation–demethylation). These processes enable cells to communicate with their environment, and allow them to respond to changing conditions such as pH, osmolarity, pressure, temperature.
- uptake of ions and nutrients (mostly molecules of lower molar mass) and the secretion of metabolites and other smaller molecules (e.g. signalling molecules, siderophores) depend on different types of transport systems, which are either using primary energy sources such as ATP or which are coupled to a gradient like the membrane potential.
- transport (import and export) of polymers, including proteins, is also mediated by special transport systems which, in many cases, represent multicomponent systems.

All these transport processes are of comparable importance for an organism in order to adapt to changing conditions and to exist in a given environment. This book focuses on the mass transfer aspects across biomembranes, involving ions, molecules, and particles.

Intact membranes are essential for a great variety of vital functions, such as energy-generating processes taking place in the mitochondria of eukaryotes (see above) or at the cytoplasmic membrane of bacteria. In addition, membranes are indispensable for components involved in electron transport chains, and photosynthesis is strictly coupled to the lipid bilayers. Export machineries for proteins, as well as secretion systems for a variety of substances (such as metabolites, signalling molecules, enzymes and extracellular structures) are located in membranes. Moreover, components involved in cell growth and cell division are specifically associated with membranes. In bacteria, a great variety of extracellular structures are anchored in the membranes, which constitute the envelope. Some structures (e.g. pili, fimbriae) take part in cell–cell interaction, adhesion to surfaces, and biofilm formation, others (e.g. flagellae) allow locomotion and mobility. Since so many functions and processes all occur either within, or coupled to, lipid bilayers, it is easy to realise that a fine-tuned balance of embedded and associated components is highly important for the integrity and functionality of all the different types of biomembranes. Therefore, the design and interpretation of test systems and *in vitro* assays for studying phenomena related to membranes must consider that both the elimination or overproduction of a single membrane protein (or indeed any set of components) may disturb a fragile system and lead to artificial results.

The various aspects mentioned above can be summarised as follows: fluxes and distribution of solutes in aqueous solutions and at (or through) hydrophilic/hydrophobic interphases (see Figure 4) can be modelled by following the rules of physics and physical chemistry. In many cases, equations describing transport phenomena can be rather simple, so long as processes like diffusion and osmosis are dominant, and the shapes and surfaces of particles or cells are not very complicated. However, the complexity of the situation increases greatly once parameters like 'transport against a concentration gradient', 'multicomponent systems', 'high or low affinity to a substrate' or 'complex structures associated with a cell surface' have to be taken into account. Thus the development of mechanistic concepts and models for the transport and compartmentalisation of chemicals in bioenvironmental systems requires an integrated approach, which provides functional links between processes at different levels of organisation. Indeed, a thorough understanding of environmental processes can only be achieved if studies of the chemistry (e.g. reaction kinetics and mobilisation) and the biology (e.g. transport near and across biological interphases) are combined. Although it is now widely recognised that integration is the way to proceed, these areas of research have to date been the subjects

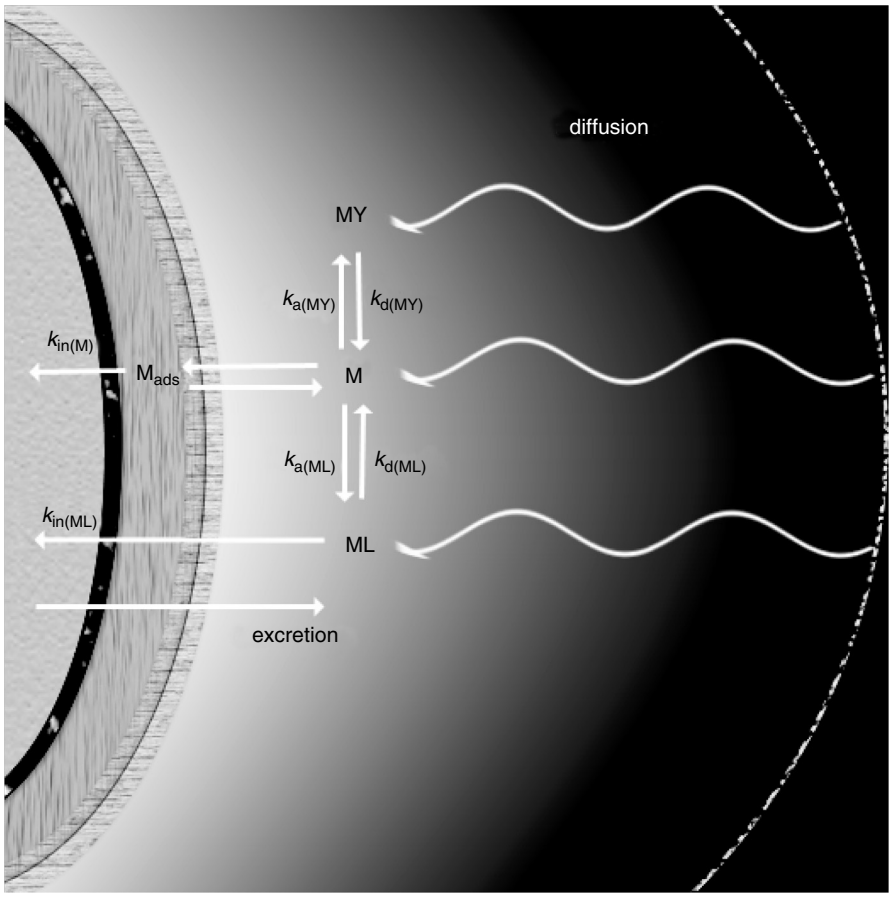

Figure 4. Schematic representation of the various processes involved in the transfer of metal ions from a complex medium to an organism. The free metal ion and the lipophilic complexes ML are effectively bioactive. Bioinactive complexes MY, present in the medium, can only contribute to biouptake processes via dissociation into M

of the individual disciplines, with little interaction between them. The present book critically summarises and integrates current knowledge of the physico-chemical mechanisms, kinetics, transport and interactions involved in processes at biological interphases in environmental systems. It starts with fundamental chapters on the physical chemistry of the structure and permeation properties of the lipid bilayer membrane (Chapter 2), and the basic features of various chemical gradients at the biological interphase and ensuing mass transport from/towards its environment (Chapter 3). The coupling of transport processes in the medium with the actual transfer of chemical species through the cell

membrane, whether or not this occurs via an adsorbed intermediate, is analysed in Chapter 4 and the role of the chemical speciation of both organic compounds and metals at the biological interphase is discussed in Chapter 5. The biochemical background of transporter functions for the transfer of chemical species across the biological membrane is highlighted for prokaryotes (Chapter 6) and for eukaryotes (Chapter 7). The particular case of transfer of colloids and particles across the biological membrane, known as endocytosis, is reviewed in Chapter 8. The active mobilisation of components in the medium by specific chemical strategies of organisms, with emphasis on mobilisation of organics, is evaluated in Chapter 9. Finally, a number of elements of the foregoing chapters are integrated in Chapter 10, where experimental data for the biological uptake of trace elements from aquatic media are modelled on the basis of knowledge of the speciation and transport parameters of the medium and the cell membrane.

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