Translated from *Uspekhi Khimii*, **57**, 1042-1063 (1988) U.D.C. 541.143

Bacteriorhodopsin as a Possible Element of Membrane Bioreactors

A.V.Maksimychev and S.K.Chamorovskii

Certain approaches to the creation of membrane bioreactors, representing an example of integrated membrane systems, are examined. The characteristic features of the use of organised molecular assemblies in such systems as sensor and regulatory elements are discussed. The properties of the retinal-protein complex of bacteriorhodopsin as a promising component of integrated membrane systems, capable of carrying out regulatory functions, are examined.
The bibliography includes 139 references.

CONTENTS

I. Introduction 592
II. Membrane bioreactors 592
III. Functional properties of bacteriorhodopsin in artificial systems 597

I. INTRODUCTION

The application of membrane technology in modern industry makes it possible to economise on resources and energy and to reduce greatly the influence of unfavourable factors on the environment. In recent years a tendency has arisen in membrane research to combine various processes, since "integration" of technological stages makes it possible to make the best use of the potential advantages of membrane systems. This procedure represents the embodying of the principles constituting the basis of the functioning of biological systems, which are distinguished by the specificity and interdependence of processes, the combination of a number of functions in a single element, and the existence of regulatory mechanisms at various stages of the process.[1] An excellent example of integrated photochemical processes is natural photosynthesis. It includes the collection of solar energy by the chlorophyll of the antenna and by other pigments, the transfer of the excitation energy to the reaction centre, the separation of charges in the photosystems I and II, electron transfer, and other processes.[2] The concept of "integrated membrane systems" presupposes not only the application of various membranes in successive separation stages, but also the combination of functions, for example, the coupling of chemical reactions with membrane separation, the incorporation in the system of membrane sensors for the control of the dynamics and optimisation of the processes, etc.[3] It may be expected that the combined application of membrane systems will prove most effective in fields such as agroindustry, petrochemistry, biotechnology, and the processing of sewage.

II. MEMBRANE BIOREACTORS

1. Membranes in Chemical Reactions

One of the vigorously developing branches of membrane technology involves the creation of membrane reactors (MR) in which the reaction products (or one of the products) are separated from the reaction mixture. The advantages of MR are manifested particularly in carrying out reversible reactions: the continuous removal of the products shifts the reaction in the forward direction and makes it possible to achieve a high degree of conversion of the starting materials. One of the possible ways of carrying out such a process is associated with the use of selective gas separation membranes.[4,5] A schematic illustration of a MR of this type is presented in Fig.1. (Since questions concerning membrane catalysis are considered in detail in another review in the present issue of the journal,[1] we shall confine ourselves to only certain examples). The non-equilibrium nature of the process in this type of flow MR with a microporous glass membrane made it possible to increase the degree of conversion of cyclohexane into benzene by a factor of ~2.5 compared with the equilibrium value. The advantages of the MR are manifested particularly clearly when a palladium membrane, permeable exclusively to H_2, is used.[4] In this case the degree of conversion of cyclohexane reaches 99.5% and, when the flow rates of the starting material and inert carrier gas are suitably selected, it can be increased still further. Thus benzene becomes virtually the only component at the outlet from the MR, which eliminates the necessity for the separation of benzene and cyclohexane, reducing considerably thereby the cost of the industrial manufacturing process.

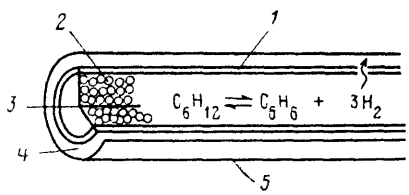


Figure 1. Schematic illustration of a membrane reactor with a palladium membrane for the dehydrogenation of cyclohexane:[4] 1) palladium membrane; 2) catalyst; 3) cyclohexane; 4) inert carrier gas; 5) shell.

Another most imporatant trend in the development of the MR is the study of the properties of catalysts stabilised on polymer membranes. In this case the membrane plays the role of a medium in which a high local concentration of the reactants and the necessary

environment can be created. A large number of studies have been devoted to the photocatalytic decomposition of hydrogen sulphide in the presence of colloidal CdS immobilised on a fluorine-containing cation-exchange membrane of the Nafion type.[6-8] The internal structure of membranes of this type simulates the structure of the inverted micelles, because it includes ionic structures connected by channels and incorporated in the hydrophobic fluorocarbon phase.[9,10] Cationic substrates sorbed in the membrane channels from the aqueous phase are acted upon by a powerful electric field,[11] which apparently must be taken into account in considering the catalytic properties of the complexes. The high chemical stability of the perfluorinated polymer membranes makes it possible to employ them in corrosive media over a wide temperature range. The photodecomposition of H_2S has aroused interest in connection with the search for non-traditional energy carriers, to which the molecular hydrogen obtained as a result of the reaction belongs. Furthermore, it is possible to reduce in this way the effect of the environment by toxic sulphur compounds, many tonnes of which are evolved as waste in chemical, metallurgical, and petrochemical industries.[6] Finally, elemental sulphur, the secondary reaction product, is a valuable raw material and natural gas deposits with a high hydrogen sulphide content may serve as its source.

Another possible application of membrane photocatalysts for the solution of ecological problems is associated with the purification of sewage. The photodegradation of chlorophenols in aqueous solutions, activated by doping with polyphenylacetylene immobilised on a poly(vinyl chloride) membrane, has been investigated.[12] The immobilisation of polyphenylacetylene makes it possible to stabilise it, preventing, in particular, the liberation of the doping agent. The rate of photodecomposition of organic impurities increases significantly with increase in the electrical conductivity of the membrane. It has been suggested that the mechanism of the photodegradation includes the formation of intermediate radical states, while the presence in the membrane of an organic conductor with electronic conductivity permits photoinitiation and accelerates oxidation-reduction processes.

2. Membrane Bioreactors

The development of MR in which chemical reactions take place under the influence of catalysts of biological origin has expanded greatly in recent years. Apparatus of this kind is referred to as membrane bioreactors (MBR). The solution of a set of problems associated with the application of MBR, including the manufacture, isolation, and purification of enzymes and their use as immobilised biocatalysts, is nowadays a leading branch of biotechnology. Membranes serve as a cheap and reliable base for the immobilisation of enzymes and for the creation of compact and flexible devices in which the separation process is coupled with a chemical reaction. Biocatalysts are placed on one side of a suitable membrane, the substrate is brought into contact with the biocatalyst, and the resulting product, whose molecules must be fairly small, permeates through the membrane. This general principle of the design of MBR has been applied in the following main types of MBR used, differing in configuration and the reaction conditions:[13] (1) membrane modules combined with a reaction compartment which contains the biocatalyst in a dissolved form; (2) reactors with membranes in the form of hollow fibres in which the enzyme is usually located on the outer side of the membrane, while the substrate circulates through the inner space within the fibres; (3) reactors with a membrane made of a mouldable solution containing the biocatalyst; (4) reactors with the enzyme fixed by cross-links on a microporous film or grafted covalently to the latter.

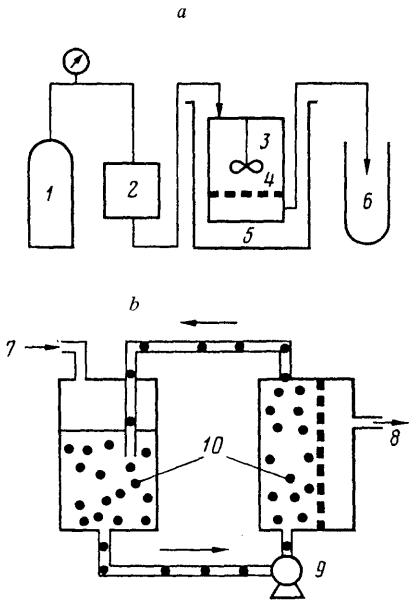


Figure 2. Membrane bioreactors with continuous mixing:[18] *a*) direct filtration reactor: 1) gas cylinder for the establishment of the transmembrane pressure; 2) compartment with the substrate; 3) reaction vessel with stirrer; 4) membrane; 5) thermostatted compartment; 6) vessel for the product; *b*) membrane flow bioreactor: 7) initial mixture; 8) product; 9) pump; 10) biocatalyst.

The appearance of MBR of the first type became possible after the creation in 1965 of the ultrafiltration membrane,[14] which was used to concentrate enzymes.[15,16] In 1970 the first membrane bioreactor was constructed in which a solution of α-amylase from *B.subtilis* was used to hydrolyse starch.[17] A considerable proportion of the enzymes used as biocatalysts have molecular weights ranging from 10 to 100 kDa and are retained by membranes designed for ultrafiltration. Reactors of the first type operate under ideal mixing conditions and are most suitable for reactions inhibited by the product, since the latter is continuously removed as it is formed, which increases the yield. Two varieties of ideal mixing MBR, differing in the organisation of the substrate and product streams are known (Fig.2).[18] Fig.2*a* presents a scheme for a MBR operating under direct filtration conditions. A significant disadvantage of such MBR is the contamination of the membrane and also concentration polarisation, the elimination of which requires vigorous stirring of the contents of the reaction compartment. Reactors constructed in accordance with the above scheme are used mainly for laboratory research. A flow MBR free from the above disadvantages was first proposed by Porter and Michaels[19] (Fig.2*b*). In contrast to the scheme in Fig.2, the separating membrane module is in this case physically separated from the reaction vessel containing enzymes or whole cells as the catalyst. The flow MBR makes it possible to use

the biocatalyst repeatedly and to organise the operation of the system under stationary conditions. Reactors of this type are suitable for industrial use, for example, in apparatus for the treatment of sewage.[20,21] For the normal functioning of MBR of the type considered, it is essential that the product molecules should be significantly smaller than the substrate and biocatalyst molecules. By virtue of this limitation, MBR of the given type have found applications mainly in the hydrolysis of proteins, starch, and cellulose.[18] Low concentrations of the components and relatively low rates of conversion are characteristic of the MBR operating under ideal mixing conditions.

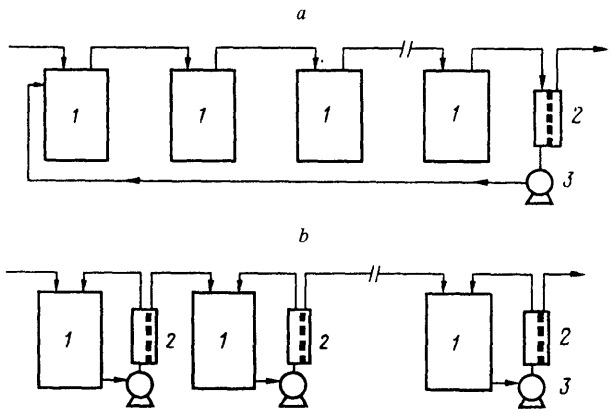


Figure 3. Cascade membrane bioreactors with modules for membrane separation in the concluding stage (*a*) and in each stage (*b*): 1) reactor; 2) membrane separation unit; 3) pump.[18]

The search for highly productive systems, in which a high degree of conversion of the starting material is attained, led to the creation of cascade MBR (Fig.3).[18] Two types of cascades are used: with the membrane separation unit in the concluding stage (Fig.3*a*) and with recirculation of the unreacted component at each stage (Fig.3*b*). Cascade systems of the later type are fairly expensive but ensure a high flexibility of the system, since they create the possibility of carrying out each stage under optimum conditions, which is especially convenient for multienzyme processes.

A higher degree of integration of the chemical reaction and the membranes separator is achieved in MBR based on hollow fibres, since in such devices the membrane separation cell also functions as the reactor. The principal advantage of devices based on hollow fibres is a high ratio of the membrane's surface area to the volume which it occupies. Furthermore, the operating conditions for such MBR approach the ideal displacement regime, which permits the attainment of a high degree of conversion of the substrate. A MBR based on commercial hollow fibres in which an enzyme is enclosed was first described by Rony.[22] Subsequently different versions of hollow fibre MBR, representing two main configurations (Figs.4*a* and 4*b*), were proposed. In the scheme illustrated in Fig.4*a*, the enzyme is deposited on the outer surface of a hollow fibre and the substrate flows through its interior. The products can be removed either from the outer side or from the inner volume within the fibre. Preliminary ultrafiltration of an enzyme solution or a cell suspension through the membrane is widely used for the deposition of the biocatalyst on the surface of the hollow fibre. For example, yeast cells have been deposited in this way on the outer surfaces of ultrafiltration hollow fibres.[23] The cells are then fixed quite firmly in order to ensure the operation of the MBR in the course of ~12 days. The yield of the reaction product is directly proportional to the amount of cells charged into the reactor, but, for a high charge density, there is a sharp increase in the pressure necessary to maintain an acceptable rate of passage of the substrate. An appreciable increase in the transmembrane pressure is undesirable, since under these conditions the biocatalyst is washed off the fibres at a much higher rate. The method of fixation of enzymes by means of physical adsorption has many advantages: a simple and universal deposition procedure, the reduction to a minimum of the steric hindrance in the microenvironment of the enzyme, which is accompanied by an insignificant loss of its activity, and also the absence of the necessity for the introduction of any additives to the biocatalyst, which facilitates the formation of sterile preparations.

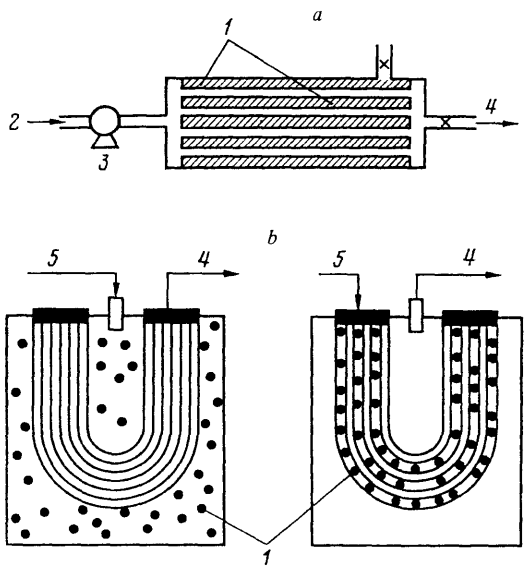


Figure 4. Principal configurations of bioreactors with membranes in the form of hollow fibres: *a*) bunch of fibres in a shell; *b*) bunch of fibres in a compartment containing the biocatalyst or serving for the removal of product; 1) biocatalyst; 2) initial mixture; 3) pump; 4) product; 5) substrate.[18]

The hydrolysis of triglycerides with formation of aliphatic acids and glycerol belongs to the class of technically important reactions. This reaction has been achieved[24,25] in MBR constructed in the form of a module with hollow aromatic polyamide fibres. The hydrolysis of triglycerides from vegetable oils occurred in the presence of lipase from *Candida cylindracea* immobilised on the inner surfaces of the hollow fibres. The enzyme was immobilised by the preliminar ultrafiltration of an aqueous solution of the lipase through the hollow fibres. The substrate—a vegetable oil emulsion—was supplied continuously into the interior of the hollow fibres. The polyamide

membrane is permeable to only one of the reaction products – glycerol, which diffused into the aqueous phase on the outer side of the fibre. Aliphatic acids were removed from the MBR as part of the composition of the lipid phase. The polyamide fibre ensures an effective separation of the phases without their dispersion in any way. Hollow fibre MBR permit the attainment of ~90% conversion of the substrate in 2–3 h,[25] and the time of the stable operation of the system is more than three weeks. A MBR of analogous design has been used for the large scale preparation of 6-aminopenicillanic acid.[26]

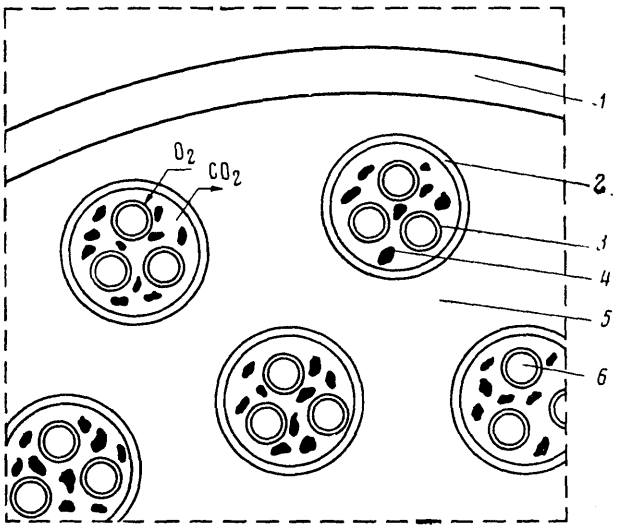


Figure 5. Cross-section through a bioreactor with a double hollow fibre:[28] 1) glass tube; 2) silicone tube; 3) polypropylene hollow fibre; 4) immobilised cells; 5) gas phase; 6) liquid phase.

One of the limitations imposed on the employment of hollow fibre MBR is that the substrate and product must have a low molecular weight, permitting their diffusion through the membrane. This is why such reactors are frequently used for the hydrolysis of oligomers.[18] Another main disadvantage of apparatus based on hollow fibres, already noted in the first relevent investigation,[22] is the limitation of the rate of the chemical reaction by diffusion processes. For example, when MBR with hollow fibres made of polypropylene were used for the production of phenylalanine by mutant strains of *E.coli*,[27] it was observed that the rate of the chemical reaction is limited by the diffusion of oxygen, necessary for the metabolism of the above cells. It has been suggested that reactors of this type are more suitable for systems with a low consumption of oxygen or when anaerobic organisms are employed. Analogous difficulties arise also when gases are evolved in the course of the enzymic reaction. The attempts to overcome these limitations led to the creation of MBR based on double hollow fibres.[28] Fig.5 illustrates schematically the design of a double hollow fibre bioreactor suitable for the cultivation of aerobic cells. The micro-organisms are located in the space between silicone tubes through the walls of which oxygen permeates and hollow polypropylene fibres through which the liquid phase passes. The productivity of the MBR based on double hollow fibres can exceed by a factor of 20–30 that of the traditional enzyme reactors.

MBR are known in which the biocatalyst is enclosed in a porous polymer matrix.[13] The biocatalysts may consist of enzymes or whole cells resistant to the action of the organic solvents used in the formation of the membrane. Many bioreactors of this type have been developed for the processes used in the food industry. In order to combine in a single cell ultrafiltration and the hydrolysis of lcatose in milk and whey, a planar version of the MBR has been proposed.[13] Lactose is immobilised in a microporous poly(vinyl chloride) film placed directly behind the ultrafiltration membrane (Fig.6). The coupling of the processes makes it possible to avoid the undesirable contact between the enzyme and particles of milk fat. An analogous process has been achieved in the presence of the bacteria *Sulfolobus solfataricus* in a MBR of a different geometry.[29] The biocatalyst was incorporated in a membrane matrix made in the form of a hollow fibre consisting of a mixture of polysulphone, polyvinylpyrrolidinone, and *NN*-dimethylacetamide. An increase in the content of lyophilised cells in the mixture impaired the mechanical properties of the fibres and reduced the regularity of the membrane structure. However, these negative effects can be compensated for by varying the molecular weight of polyvinylpyrrolidinone. The study of the relation between the functional characteristics of the given MBR and their kinetic and hydrodynamic parameters showed that they can operate under both diffusional and kinetic conditions depending on the conditions specified at the inlet.

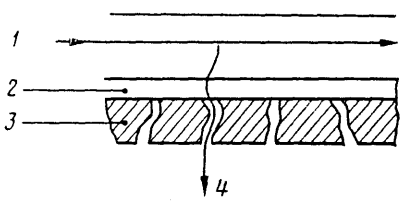


Figure 6. Schematic illustration of a flat membrane reactor with immobilised enzyme:[13] 1) substrate; 2) ultrafiltration membrane; 3) film with immobilised enzyme; 4) hydrolysate.

The microenvironment influences significantly the activity of the biocatalyst incorporated in the polymer matrix. The mechanical properties of the polymer, its biocompatibility, and its resistance to decomposition by bacteria play an important role.[30]

Membrane bioreactors in which the enzyme is immobilised as a result of the formation of cross-links or as a result of covalent attachment to the carrier surface have many advantages: they are stable, make it possible to organise the process under continuous and controlable conditions, and it is possible to obtain a uniform and relatively cheap product.[18] However, depending on the method of immobilisation, different enzymes can lose between 10 and 90% of their activity in a free state as a consequence of steric hindrance and diffusional limitations. One of the ways of overcoming the diffusional control of the chemical process in the MBR is based on the application of an asymmetric ceramic membrane.[31] Invertase is immobilised in such a membrane by filtration through a porous support and subsequent treatment with glutaraldehyde. The high

mechanical strength of the membrane makes it possible to carry out the chemical reaction under filtration conditions at pressures in the range 50–450 kPa. The contact time between the enzyme and the substrate depends on the pressure, whose variation makes it possible to regulate the rate of the reaction. The elimination of diffusional limitations ensures a significant increase in the flow of substance through the membrane.

The investigations carried out assisted in the development of a large number of a wide variety of MBR designs and demonstrated the wide scope of their uses. Among the fields in which the application of MBR may prove very effective, we may specify the preparation of ecologically pure and renewable energy carriers. The last 15 years have been characterised by a sharp increase in the employment of alcohol-containing automobile fuels which in certain countries have already acquired practical importance.[32,33] Analysis shows[32] that the coupling of fermentation with processes involving reverse osmosis, ultrafiltration, and evaporation through a membrane makes it possible to improve the economic parameters of the manufacturing process and to justify the wide-scale employment of butanol, ethanol, and acetone as fuels. The designs of various membrane fermenters,[34,35] including those for the synthesis of ethanol,[18] are being vigorously developed.

Methane and molecular hydrogen are believed to be a promising basis for future power engineering. One of the possible ways of obtaining H_2 – via the photodecomposition of hydrogen sulphide – was mentioned previously. An alternative approach is based on the fact that hydrogen and methane are the products of a large number of micro-organisms,[33,36,37] including phototropic micro-organisms for which light serves as the energy source. Hydrogen can be evolved both by whole cells and by isolated polyenzyme systems containing hydrogenases,[38] the activity of the hydrogenases exceeding appreciably the activity of the catalysts based on platinum group metals and persisting for a long time at a high level (the half-inactivaion period is about 60 days[39]). Thus the creation of fermenters for the manufacture of hydrogen and methane in which the principles of MBR would be realised (see, for example, Fig.5) is of undoubted interest for the solution of energy problems. The survey of modern concepts in the design of MBR for plant cells has been published.[40] A favourable factor for the development of hydrogenase-containing MBR is also the fact that, when hydrogenases are incorporated in lipid liposomes (a procedure used for the immobiliisation of biocatalysts on synthetic membranes[41]), an increase not only in the stability but also in the activity of the enzyme is observed in a number of instances.[42]

3. Organised Molecular Assemblies

Organised molecular assemblies – structures formed spontaneously by amphiphilic molecules in aqueous solutions – are of considerable interest for the development of integrated membrane systems. The simplest organised molecular assemblies are monolayers on interfaces, and micelles, microemulsions, double layers, and vesicles.[43] The tendency towards self-organisation by lipid molecules in the form of a double layer determines the principal structural features of biological membranes. Liposomes, closed double layer lipid membranes containing an internal aqueous phase with dimensions in the range 20–500 nm, as well as molecular monolayers have attracted most attention. The use of liposomes to carry out chemical reactions is determined by the following main factors.[41,43] Firstly, in the inner volume and in the walls of the liposome, the test substances are present at relatively high concentrations. Secondly, the formation of liposomes is accompanied by the creation of a surface with an extremely large area. For example, at a lipid concentration of 4 mg ml^{-1}, the area of liposomal membranes is ~ 1 m^2 ml^{-1}. Thirdly, liposomal membranes are characterised by a high permeability to non-electrolytes (10^{-4}–10^{-6} cm^3 cm^{-1} s^{-1}) and a low permeability to hydrophilic molecules (10^{-9}–10^{-10} cm^3 cm^{-1} s^{-1}). A liposome containing in the inner aqueous phase some kind of enzyme is in essence a membrane microreactor constructed at the molecular level.[44] Liposomes can be used in MBR for the soft immobilsation of enzymes:[41] initially the enzyme is built into the liposomal membrane, where it is incorporated in the inner aqueous phase, and the liposomes are then immobilised on a porous membrane. The stability of the immobilised liposomes is higher compared with the free liposomes. Another way of increasing stability consists in creating around the vesicle a polymer shell chemically bound to the outer surface of the membrane.[45] The vesicles, which are stable in the course of several months, can be prepared from molecules with double bonds which undergo polymerisation after the formation of the vesicles.[43]

Microcapsules, which are analogous to polymeric vesicles but are larger, have been investigated as "containers" for biocatalysts in pioneering investigations.[46,47] Microcapsules having diameters ranging from 1 to 100 μm have been obtained by interfacial polycondensation, aqueous solutions of the enzymes being emulsified in an organic phase. A solution of the enzyme, whole cells, or their fragments were enclosed in nylon or collodion capsules, no appreciable leakage of the protein from the capsules being observed in the course of approximately a week. The membranes of the capsules are permeable to low-molecular-weight substrates and products, their transport taking place by means of diffusion via water pores ~ 1.6 nm in diameter, and in the most successful specimens the activity of the enzyme, measured as the rate of accumulation of the product, approaches the activity in the native state.[46] The possibility of using microcapsules in biochemical studies *in vitro* and also for therapeutic purposes *in vivo* has been studied. One of the significant limitations in the use of systems of this kind consists in the risk of the denaturation of the protein in interfacial polymerisation in the course of the formation of the microcapsule. Earlier studies on the encapsulation of enzymes served as a basis for the development of the first MBR containing the biocatalyst within hollow fibres.[22]

The development of studies on microencapsulation led to the creation of systems in which interfacial catalytic reactions can be carried out at a liquid/liquid interface. The enzyme is immobilised on the outer surface of a porous nylon capsule filled with an aqueous buffer solution,[48] which is placed in the organic phase (Fig.7). The synthesis of peptides takes place at the interface between the aqueous phase and chloroform, the enzyme being protected from the inactivating effect of the organic solvent by a surface layer of buffer solution seeping through. In contrast to the traditional capsule MBR, in this bioreactor, the molecular weights of the starting materials and products need not necessarily be low. Other advantages of the system are the simplicity of the separation of the biocatalyst from the substrate and product and also the absence of an induction period in the catalytic reaction associated with swelling processes.[48]

The use of an organised assembly of molecules in the form of a monolayer at the interface ensures major technological possibilities. The method of depositing monomolecular layers on the surface of a solid support proposed by Langmuir constitutes nowadays the basis of numerous developments in the field of microelectronics, optics, and electro-optics and for the creation of photoelectric converters and information processing systems.[49] The introduction of methods

for the preparation of ultrathin layers into membrane technology makes it possible to outline new approaches to the creation of integrated systems.[50] In the first place, one should note the possibility of obtaining infinitesimally thin composite membranes which could be used to devise highly productive MBR. The high degree of order in the molecular arrangement in the monolayer makes it possible to carry out chemical reactions under unusual conditions where highly specific effects can be manifested. The successive deposition of different monolayers permits the creation of mixed structures whose properties can be controlled at the molecular level with attainment of maximum integration. The introduction of suitable functional components into a thin layer system renders them sensitive to chemical, electrical, or optical signals, which can be used to control properties of the deposited film. In such systems, one can achieve not only material transfer but also transfer of information. Thus the study of integrated membrane systems requires the expansion of traditional ideas about the membrane as a passive selectively permeable partition, because in systems of this kind it can play the role of an active element acting on the chemical state of the given substance (membrane reactors) or participating in the generation and transformation of signals (membrane sensors).

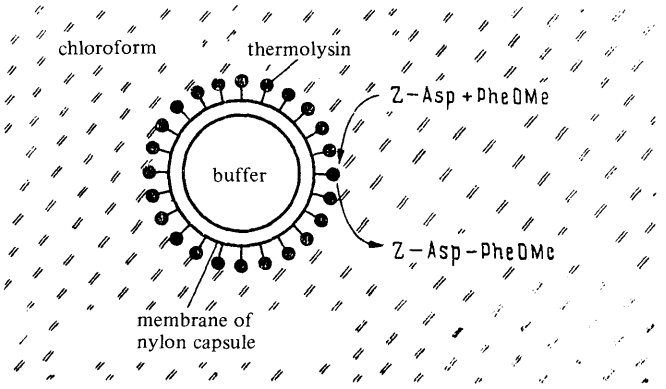


Figure 7. Design for a capsule membrane bioreactor for the synthesis of peptides.[48]

We shall deal briefly with certain examples of the use of thin-layer membrane systems. A method has been proposed for the formation of a thin-layer membrane from fragments of cell walls of certain bacteria.[51] The immobilisation of glycoprotein fragments having pores of uniform size 2–6 nm in diameter on the surface of a porous synthetic support makes it possible to use the resulting membrane for ultrafiltration in the pH range from 1 to 13.

Studies on processes involving the photoinitiated intramolecular electron transport in ordered donor–sensitiser–acceptor systems, formed by the Langmuir–Blodgett method, showed that it is possible to achieve states with separated charges on a nanosecond time scale.[50] A system functioning as a photodiode has been obtained by depositing on a gold electrode monomolecular films containing bound residues of viologen, pyrene, and ferrocene.[52]

Thin-layer membranes have found extensive applications as sensitive elements in chemical sensors for biomedical research and for the control of processes in bioreactors *in situ*.[53] One of the ways of creating sensor devices consists in the use of the structure of a semiconducting field transistor as the base for the deposition of a membrane exhibiting a specific chemical sensitivity.[53–55] Planar silicon transistors with an oxide or nitride dielectric film are usually employed, the immobilised enzyme being deposited on the region of the transistor gate. The change in the electrical potential at the gate as consequence of the chemical interaction with the substrate modulates the state of the conducting channel, which is recorded by electrical methods. This principle has been used to create biosensors sensitive to glucose, ATP, glutamic acid, urea, and other organic compounds.[53–55] Biosensors of this type have many undoubted advantages: they can be miniaturised, are readily "integrated" in technological schemes, make it possible to combine various sensors in a single semiconductor crystal, have good metrological characteristics, and there exists a sophisticated technology for their preparation. The use of enzymes in biosensors ensures the possibility of recording a broad spectrum of substances with an enormous sensitivity (the concentration of the substances detected can be as low as 10^{-19} M).[56] The use of thin-layer membranes in sensor devices is justified by the fact that the response time of the sensor is inversely proportional to the square of the thickness of the sensing membrane and can amount to several seconds.[54]

Membranes based on double-layer and multilayer molecular structures have been used successfully for the highly selective determination of anions and cations by electrochemical[57,58] and spectroscopic[59] methods. A monomolecular lipid layer at the water/air interface has been investigated as a model of the olfactory membrane.[60] The absorption of a gaseous component (diethyl ether) or its incorporation in the lipid monolayer increases sharply the permeability of the film to water vapour.

Photo- or chemo-sensitivity of the membranes can be used not only for the creation of recording systems but also for the active regulation of the functional properties of membranes in integrated systems. The possibility of a controlling effect on the components of complex membrane systems greatly extends the number of independent operational variables and permits the realisation of new operating conditions and an increase in the flexibility of such systems, for example, membrane bioreactors.

One of the biological objects having a wide set of functional properties which can find technical applications is the membrane protein complex bacteriorhodopsin. The principal properties of bacteriorhodopsin and the possibilities for its employment in membrane systems are considered below.

III. FUNCTIONAL PROPERTIES OF BACTERIORHODOPSIN IN ARTIFICIAL SYSTEMS

1. Purple Membranes Containing Bateriorhodopsin

It is well known that lipid membranes play a key role in the processes involving the transformation of energy by living cells. In the highly specialised biological membranes effecting fundamental processes such as respiration or photosynthesis, the multienzyme complexes bound to the membrane catalyse a whole chain of coupled electron- and proton-transfer reactions in the course of which chemical energy is stored.[61]

Recently the so called purple membranes (PM), synthesised by the bacteria *Halobacterium halobium*, have attracted the attention of investigators. In these comparatively simple but highly specialised

membranes, a vector transmembrane transfer of H^+ takes place under the influence of light.[62]

The purple membranes from *H. halobium* consist to the extent of ~75% of a single protein—bacteriorhodopsin (Br),[62] while the remaining 25% comprises acid lipids.[63] Bacteriorhodopsin is a single chain protein consisting of 248 aminoacid residues[64,65] and having a molecular weight of ~26.5 kDa. The chromophoric group of the protein is the aldimine of retinal, which is bound covalently to the ε-amino-group of lysine, forming a Schiff base.[62]

The X-ray low-angle and diffraction methods[66] as well as limited proteolysis and immunochemical binding to monoclonal antibody experiments[67] have shown that the polypeptide chain of the Br molecule has seven α-helical transmembrane strands connected by protein sections exposed to the aqueous phase on the surface of the membrane. According to electron microscope data, the Br molecules in the plane of the membrane form a dense hexagonal quasi-crystalline lattice,[68] whose main structural element is Br trimers (Fig.8).

The Br in the membrane can exist in two stable forms—the dark-adapted and light-adapted forms between which transitions are possible. In the light-adapted Br, the retinal is in the fully *trans*-conformation, while the dark-adapted Br is an equimolar mixture of 13-*cis*- and fully *trans*-isomers.[69] Both 13-*cis*-Br and the fully *trans*-Br are photochemically active and are characterised by cycles of photochemical transformations.

The photochemical cycle of the light-adapted fully *trans*-Br has been studied in fair detail from the primary sub-picosecond stages. During the cycle, deprotonation and subsequent reprotonation of the Schiff base and the isomerisation of retinal with respect to the C(13)=C(14) double bond take place. During the photocycle, the fully *trans*-Br passes through at least six metastable states—the intermediates J, K, L, M, N, and O, whose lifetimes are in the range 0.4 ps–(5–10) ms (see the relevant reviews[70,71]).

Very much less is known about the photocycle of 13-*cis*-Br, for which three intermediates have been postulated: ^{x}C, ^{y}C, and ^{610}C (610 nm is the maximum of the optical absorption of the intermediate). In the ^{y}C state the photocycle branches and there is a probability of the transition of Br to the fully *trans*-form.[72]

The nature of the primary intermediate in the photocycle has continuously attracted the attention of investigators, because this is in fact the site where energy is stored and is subsequently used for the transmembrane transfer of H^+.

It has been established with the aid of pulse laser absorption and fluorescence spectroscopy with sub-picosecond time resolution that, after the absorption of a light quantum, the light-adapted Br passes to a singlet excited state, whose lifetime is τ = 0.4–0.7 ps.[73] The rate of relaxation of the excited state is the same as the rate of formation of the intermediate J, the absorption spectrum of which has undergone a bathochromic shift of ~30 nm relative to the Br spectrum. The intermediate J is converted in its turn over a period = 5 ps into the intermediate K whose absorption spectrum has undergone a bathochromic shift of 20 nm relative to the Br spectrum.[73,74] The lifetime of the intermediate K is 1–2 μs and is the same as the time required for the formation of the photocycle intermediate having an absorption maximum near 550 nm (L_{550}). In this and preceding stages, the albumin of the retinal in Br is in the protonated state.

The next stage in the cycle of photochemical transformations of Br is the transition from the intermediate L_{550} to one of the functionally most important forms, namely M_{412}, which corresponds to the deprotonated state of the Schiff base of retinal. The intermediate M_{412} is formed ~40 μs after the absorption of a photon. The formation of the M_{412} photoproduct can be recorded by low-temperature or pulse absorption spectroscopy at the absorption band near 410 nm and also from the appearance of the 1570 cm^{-1} band due to the C=C stretching vibrations in the kinetic resonance Raman spectra.

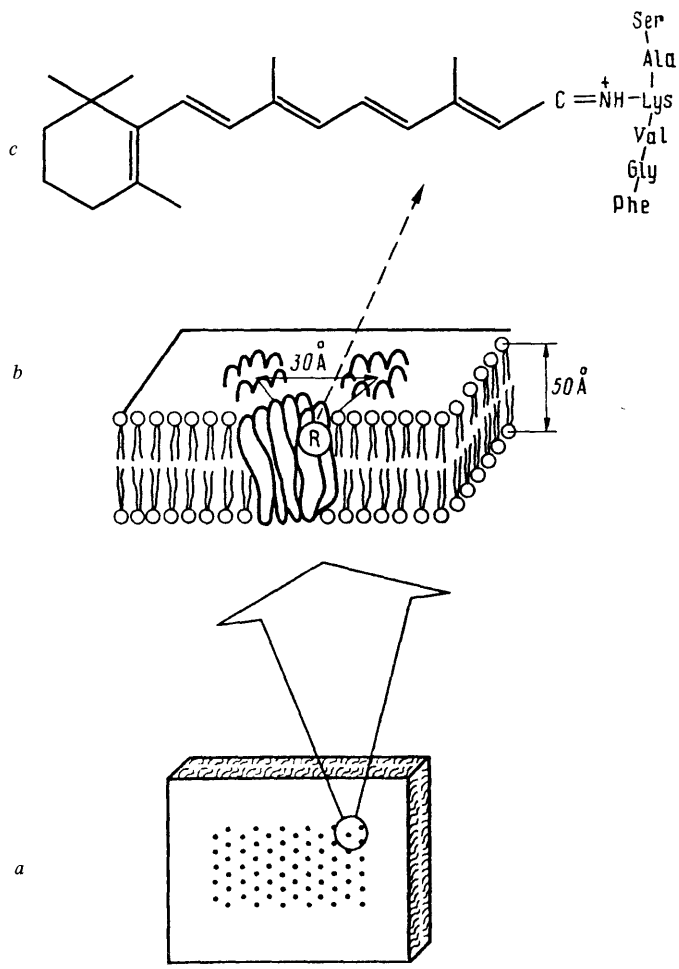


Figure 8. The structure of purple membranes containing bacteriorhodopsin:[68] *a*) fragment of the purple membrane, the dots denoting the molecules of bacteriorhodopsin forming a quasi-crystalline structure; *b*) bacteriorhodopsin trimer; *c*) chromophoric group of bacteriorhodopsin—retinal, bound as a Schiff base with Lys-216 of the polypeptide chain of bacteriorhodopsin.

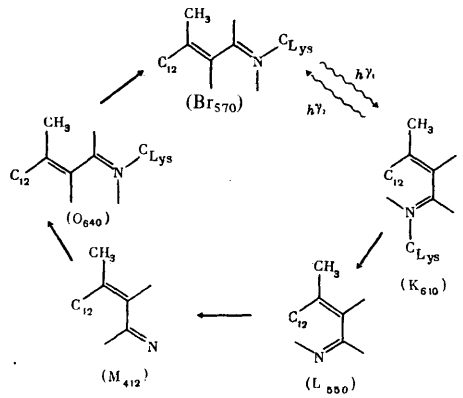
The process leading to the formation of the M_{412} form is one of the key stages in the functioning of Br as a light-dependent proton pump. The most significant changes in the ionic state of a number of aromatic aminoacid residues and carboxy-groups of the protein

have been recorded in the stages involving the formation and decomposition of the M_{412} intermediate. It has been established that a proton is split-off from the Schiff base and H+ is transferred through the membrane at this stage.

Approximately 10 ns after the formation of the M_{412} intermediate, the regeneration of the initial form Br_{570} is observed. As a rule, this transition is described by the following sequence of photoreactions: $M_{412} \rightarrow (N_{520}) \rightarrow O_{640} \rightarrow Br_{570}$ (the N_{520} intermediate is enclosed in brackets because many workers have failed to confirm its existence). The application of pulse Raman spectroscopy with picosecond time resolution made it possible to investigate the dynamics of the conformational changes in retinal. In the range 1100–1400 cm^{-1}, the Raman spectra of 13-*cis*-Br and fully *trans*-Br differ significantly. The Raman spectrum recorded several picoseconds after the application of the laser flash to the light-adapted (fully *trans*-Br) specimen resembles very closely the spectrum of 13-*cis*-Br. When the medium with H_2O is replaced by one with D_2O, an analogous spectral correspondence is observed between the "picosecond intermediate" and 13-*cis*-Br but in a somewhat different wavelength range (950–1050 cm^{-1}). The band corresponding to the -C=NH bond of the Schiff base (1646 cm^{-1}) in the picosecond time range is broadened and undergoes a shift, which may indicate a change in the electronic structure and in the direct environment of the -C=NH groups.[75,76]

Quantum-chemical analysis of the vibrational spectra with retinal-deuteriated or ^{13}C-modified in the C(15)-position, recorded by Fourier-transformation IR spectroscopy, as well as the resonance Raman spectra of the analogous Br preparations with biosynthetically ^{13}C-modified retinal have shown that the following changes in the chromophore structure take place: Br_{570} (fully *trans*) → K(13, 14-*s*-*cis*) → L(13, 14-*s*-*cis*) → M(13-*cis*) → O(fully *trans*).[77,78]

The cycle of photochemical transformations of the light-adapted Br and the structural rearrangments of retinal near the Schiff base are illustrated in the scheme below:



2. Photoelectric Processes

In the course of the photochemical reactions of Br, protons are transferred from the intracellular volume through the membrane to the outer medium, which creates a gradient of the electrochemical potential of hydrogen ions on the cell membrane. The energy stored in the form of the difference between the electrochemical potentials is used for the synthesis of ATP, membrane transport, the motion of the bacterial cell, metabolic processes, etc. The exact mechanism of the active proton transport in Br has not been established. The energetic, structural, and kinetic aspects of the functioning of the proton channel have been discussed in the literature.[79,80]

The direct experimental measurement of the photoelectric activity of Br[81,82] is based on the retention of its functional properties when it is incorporated in artificial phospholipid membranes, liposomes, or phospholipid-impregnated membrane filters separating electrolyte solutions. The action of light induces the transfer of H^+ between the membrane-separated sections and creates an electrical potential difference, which can be measured with the aid of reversible electrodes or potential-sensitive probes.

The use of a low-inertia recording system as well as pulse laser excitation permits an increase in the time resolution of the method of measurement of the photopotential down to microseconds[83] and picoseconds.[84,85] The processes involving an increase and fall in the photopotential generated by Br have several phases correlated to a first approximation with the formation and decomposition of the intermediates K, L, and M in the Br photocycle.[83] It has been suggested that the generation of photopotential in such systems is associated with the intramembrane displacement of charged groups (displacement currents) and the transmembrane H^+ transport.

Model systems containing Br as part of the composition of artificial lipid membranes can consist of liposomes or planar macroscopic membranes. It has been shown with the aid of potential-sensitive dyes that a photopotential up to 200 mV arises on the membrane in artificial lipid vesicles under stationary conditions.[86] In the liposomes, the direction of proton transport can be the same as the direction of transport in cells or can be opposed to it[87] depending on the method of preparation of the liposomes, the type of lipids used, and the pH of the medium.[88,89]

The Br in liposomes can be incorported as a single protein component or in combination with other membrane proteins, such as ATPase, cytochrome oxidase, and other enzymes of the respiratory chain.[87,90–93] In the last case, the membrane electrochemical potential, generated by Br on illuminaion, can provide the energy for phosphorylation processes and can exert a regulatory effect on the activity of enzymes. The organised molecular assemblies obtained when Br and other enzymes are incorporated in double layer lipid membranes make it possible to achieve functional coupling between proteins, the monomeric Br serving as a more effective source of energy than the native purple membranes by virtue of the closer packing of the monomer in the liposome.[94]

Br is capable of being sorbed at the octane/water interface and, under illumination in the presence of an electron acceptor in the octane phase, a potential difference up to 0.5 V is generated between the phases.[95] When ATPase is introduced into such a system, photophosphorylation can take place in the latter.

The photoelectric activity of Br in planar double-layer ("black") lipid membranes was first demonstrated in two studies.[96,97] It was established that the photopotential of 100–180 mV, arising when the system is illuminated with light at a wavelength of ~560 nm (the Br absorption maximum), diminishes to zero under the influence of blue light absorbed by the intermediate M driving the photocycle. An analogous regulation of the photoelectric properties of Br by light having different spectral composition occurs also in other model systems.

The principal disadvantage of planar double layer lipid membranes is their low stability. The addition of fillers to solutions from which the membranes are formed makes it possible to increase severalfold the lifetime of the membrane and the breakdown potential.[98] Even more stable systems are obtained when Br is sorbed on lipid-impregnated porous filters or collodion membranes.[99]

The photoelectric activity of Br immobilised in polyacrylamide gel has been recorded in a number of studies.[100,101] The kinetic characteristics of the photoelectric signals indicate that Br remains intact on polymerisation of the gel.

Br or purple membranes can be immobilised in other polymer matrices [poly(vinyl alcohol), gelatin]. Such dry membranes with additives modifying the spectroscopic and kinetic properties of Br have come to be called "Biokhrom" films. They constitute a promising photochromic material for the reverse optical recording of information.[102]

The immobilisation of Br increases the stability of this protein, which is high even in the absence of this step and is unique for biological systems. Thus Br retains its photochemical activity over a wide temperature range (from helium temperatures to ~370 K), a wide pH range (3–10), and a wide range of ionic strengths of the solution (from distilled water to saturated NaCl solution) and exhibits a high resistance to the action of proteolytic enzymes[67,103] and certain organic solvents and detergents.[104] The "Biokhrom" films or dry films prepared from purple membranes retain their funcional activity for many months and even years and withstand without loss of activity up to 10^7 "light–dark" switching cycles. For certain applications, such "solid state" systems, obtained by immobilising and drying Br on solid supports, are in fact most promising. The functioning of Br in dry membranes has a number of characteristic features compared with its functioning in the whole cell or in an aqueous suspension of purple membranes.

3. Characteristics of the Photochemical Reactions of Bacteriorhodopsin in Dry Membranes.

Br retains its capacity for reversible photochemical reacions under the conditions of partial dehydration, the reaction cycle in fairly moist films ($\gtrsim$60%) being virtually the same as in an aqueous suspension.[105,106] The formation and decomposition of the intermediate M, i.e. deprotonation and protonation of the chromophoric group of Br in the absence of water as the liquid phase, show that these processes apparently proceed in the cell with participation of endogenic proton donors and acceptors, which are probably the aminoacids tyrosine and aspartic acid localised in the vicinity of the Schiff base.[80,105]

Together with the similarity of the photochemical processes occurring in suspension and in dry purple membrane preparations, certain significant differences are observed between them: (1) for a low humidity, the position of the maximum in the absorption spectrum of multilayer purple membrane films undergoes a hypsochromic shift of 5–10 nm compared with the maximum in the spectrum of the suspension.[107] (2) The dark and light adaptation in multilayer purple membrane films of a relative humidity < 100% differs from the adaptation in the purple membrane suspension.[107,108] In the films which are in the light-adapted state, the ratio between the Br isomers with 13-*cis*-retinal and the fully *trans*-retinal depends on the wavelength of the exciting light and on the humidity. (3) Together with the photochemical reactions of fully *trans*-Pr in dry purple membrane preparations, photoreactions of 13-*cis*-Pr, from the photocycle of which the deprotonated intermediate M is absent, are observed. (4) The photochemical cycle of the fully *trans*-Br in dry membranes is reduced compared with the photocycle in an aqueous suspension – the intermediates L and O are absent[106,109,110] while in moist membranes the characteristics of the Br cycle approach those of the photocycle in cells.[111]

After extensive dehydration of the purple membrane, the absorption maximum of Br shifts from 570 to 530 nm.[112] According to Raman spectroscopic data, in the new state aldimine is in the deprotonated form, while retinal is predominantly in the 13-*cis*-configuration. Under illumination, the dehydrated Br_{530} undergoes a photochemical reaction cycle with the following stages: the formation of a batho-intermediate with a spectral maximum at 630 nm, the formation of an intermediate with a maximum at 412 nm (formation time τ = 2.5 ms), and relaxation of the latter over a period τ = 2 s to the initial form Br_{530}.

Apart from the features of the photocycle indicated above, in dry films one can also note an increase in the lifetime of the intermediate M, which amounts to several seconds and even several tens of seconds at room temperature[109,110,113] compared with several milliseconds in an aqueous suspension. The lifetime of the state M increases also after the addition of glycerol to aqueous solutions of purple membranes.[114,115]

Methods for the preparation of oriented films or membranes containing purple membranes as the working element have been developed for a more far-reaching study and more complete utilisation of the molecular photochromic and photoelectric properties of Br.

4. Methods for the Preparation of Oriented Films of Purple Membranes and the Photoelectric Properties of Such Films

Various procedures for orienting purple membranes have been described: by the centrifugation method,[116] at the air/water interface as a result of hydrophobic interactions,[117] in a magnetic field as a consequence of the anisotropy of the diamagnetic susceptibility of the protein segments,[118] on charged surfaces modified with polylysine,[119] in the electric field of an air condenser,[105] and in an electric field applied directly to the suspension.[120] Chemical cross-linking has also been used to orient the purple membranes and to stabilise the resulting structure.[121]

The orienting of purple membranes in a magnetic field[118] is based on the diamagnetic properties of the α-helical structure of the protein, which is the preferred form of organisation of the Br molecules. Exposure of a purple membrane suspension to the action of a magnetic field results in the appearance in the former of a certain degree of order which can be fixed by drying.

Oriented purple membrane films have been obtained by the deposition onto a support of monolayers formed by the Langmuir–Blodgett method.[117,122] The study of the state of the purple membrane monolayers in a mixture of purple membranes with aliphatic acids at the air/water interface made it possible to determine their dynamic characteristics and to confirm the possibility of the formation of multilayers by the Langmuir–Blodgett method.[123] Membrane systems consisting of oriented molecular purple layers, obtained by the Langmuir–Blodgett method and deposited on a thin Teflon support or another neutral carrier, have been regarded by some workers as prototypes for molecular computing elements.[124,125] This system is fairly readily available and sufficiently stable to be able to carry out rigorous quantitative measurments and at the same time the molecular organisation of the purple membrane is fairly complex, which creates a basis for the modification of its structure by controlling the photosignal.

Multilayer films, within which the purple membranes are arranged rigorously parallel to the plane of the support, can be obtained by centrifugation and sedimentation methods.[116] However, in this procedure for the formation of the film, the purple membranes can be oriented with equal probability with both its cytoplasmic and

periplasmic surface directed towards the support. The preparations obtained are distinguished by a high degree of purple membrane packing order, but the direction of the photoinduced H^+ transport in each membrane is selected at random out of the two possible directions.

A similar ordering of the film can be achieved by drying a purple membrane suspension on a glass, mylar, or mica plate. In this case, the purple membranes are spontaneously "arranged" in the plane of the suport by virtue of the much greater diameter of the purple membrane compared with its thickness (about 300 and 5 nm respectively). However, in such spontaneous sedimentation, bending of the purple membrane discs and other distortions in their structure may be observed.

The methods for orienting purple membranes based on the different properties of the cyto- and peri-plasmatic surfaces are of considerable interest. Purple membranes have a large permanent dipole moment due to the different surface charge densities on the opposite sides of the membrane. The dipole moment is ~ 140 D at pH 7.[126] The freezing of the purple membrane suspension in an external electric field makes it possible to create and fix a preferred orientation of the purple membrane relative to the applied electric field vector.[127]

In order to obtain preparations of oriented purple membranes, drops of a suspension deposited on an electrically conducting support and dried in a permanent electric field have also been used.[105] However, since the drying was carried out in the field of an air condenser, the appreciable difference between the dielectric constants of air and the purple membrane suspension precluded the attainment of an appreciable electric field strength within the suspension and, furthermore, the field inside the drop was significantly inhomogeneous. A high degree of order was not therefore attained in the purple membranes in preparations obtained by the above method.

An appreciably greater order was achieved using the electrophoretic method for the deposition of purple membranes on a flat electrode.[120] In this case, the electric field is applied directly to a deionised purple membrane suspension. It is known that purple membranes contain acid lipids,[63] which are negatively charged in a neutral medium. For this reason, purple platelets exhibit an electrophoretic mobility and at a pH of about 7 the purple membranes move towards the anode.

Analysis of the methods enumerated shows that the electrophoretic deposition and subsequent drying of purple membranes on an electrically conducting support make it possible to obtain preparations in which the Br platelets are ordered to the maximum extent. Under the influence of light, such highly oriented membrane structures generate an electric potential up to 10–11 V (for a thickness of the specimen of about 10 μm [128]). The photoelectric signal generated by Br consists of two components: the displacement current associated with the redistribution of charges within the membrane and the constant photocurrent induced by the transmembrane proton transport and coupled with electrochemical reactions on the surfaces of the electrodes.[129]

The appreciable value of the photosignal and the compactness of the system, tending to decrease parasitic electrical capacitances, made it possible to record by means of the picosecond laser technique the increase in the photoelectric signal, whose front proved to be shorter than 5 ps,[84] and also to create on the basis of purple membranes the first biological photodiode having the picosecond time resolution.[85]

An important property of the Br-containing membranes is the dependence of the spectroscopic and photoelectric characteristics on the applied external electric field, which creates a basis for controlling membrane systems.[71,130]

5. The Use of Bacteriorhodopsin in Membrane Reactors

The use of external influences for regulating the conditions in the operation of MBR reflects the tendency towards the organisation of processes in accordance with the principles applied in biological systems. Flexible rearrangeable systems apparently exhibit greater potential possibilities for their optimisation. The study of photochemical reactions in polymer films as a possible way of regulating the properties of membranes is of undoubted interest. Photosensitive polypeptide membranes can be prepared from poly-L-glutamic acid containing chromophoric *para*-rosaniline groups.[131] On irradiation with UV light, photodissociation, accompanied by the conformational transition of the polypeptide to the α-helical structure, takes place. In the dark, the structure relaxes to the initial structure and the process is thermally activated. Since the transport properties of the membrane depend on the conformational state of the polypeptide,[132] illumination can be used as a way of altering the permeability of the membrane. Apart from photodissociation, a further two photochemical processes, leading to a significant alteration of the properties of the molecules forming thin membranes, are known: *cis–trans* isomerisation and reactions involving a change in the hydrophilic properties of the head groups of amphiphiles. Photoreactions of the latter type have been investigated in multilayers of photosensitive amphiphilic molecules formed by the Langmuir–Blodgett method.[133] When the films are illuminated with near UV light, their permeability to ions diminishes, which has been attributed to the formation of a more compact film structure. The transition of the membrane to a non-conducting state is photoreversible, relaxation ocurring under the influence of visible light. Thus the system behaves as an ionic phototrigger: the absorption of small radiation doses induces a fairly appreciable change in the electric current. An interesting feature of the compounds examined is that, in relation to Br, they can play the role of a photoinhibitor, because the region corresponding to absorption by the induced UV-irradiated form of the substance overlaps the absorption band of Br.[133]

The permeability of synthetic polyvinyl polypeptide copolymer membranes, which form polypeptide microdomains, depends significantly on the pH of the medium,[134] which determines the conformational state of the polypeptide sections. For an appropriate choice of geometry and also of the method of immobilisation of the pigment, one can apparently make use of the proton transport function of Br to influence processes involving the transport of organic substrates through the membranes considered. Probably other enzymes catalysing reactions with participation of H^+ can also be regarded as possible regulators of the pH-dependent properties of synthetic membranes. Oxidation-reduction processes also influence the properties of certain membranes, for example, poly-L-cysteine membranes.[135]

The permeability of multilamellar membranes formed from amphiphiles of the liquid crystal type is a function of the electric field applied to the membrane.[136] Depending on the chemical composition of the membrane, its permeability depends either on the lateral or the transmembrane electric field. In the latter case, the photoelectric activity of Br can apparently be used to regulate the permeability of liquid crystal films.

The electric potential influences not only the passive processes involving the permeation of substances through the membrane but in certain cases determines also the activity of the biocatalyst. Thus an appreciable increase in the activity of alcohol dehydrogenase, immobilised on an electrically conducting polypyrrole membrane, with increase in the potential applied to the support has been observed.[137] The increase in enzyme activity has been attributed to

the overcoming of the diffusional restrictions for products and substrates as a consequence of the change in the overall charge on the membrane matrix. Presumably the use of Br, which possesses electrogenic properties, in systems of this type is extremely effective, because the application to the membrane of a potential of 0.7 V increased by a factor greater than 10 the activity of the enzyme.[137] Photopotentials of this magnitude can be comparatively readily obtained using oriented Br preparations. In order to regulate the photoactivity of KBr itself, it has been suggested that cationic amphiphiles, capable of forming double layers in the aqueous phase, be used.[138] These substances form bonds with the negatively charged groups on the purple membrane surface, which blocks the processes involving proton capture from the medium by Br. Another approach to the regulation of the properties of Br is associated with the development of gene engineering. The methods of gene engineering made it possible to obtain Br analogues in the molecules of which individual aminoacid residues have been replaced by others.[139] Hitherto 14 such mutants, whose proton transport activity and spectroscopic activities are different to those of native Br, have been obtained.

Thus a wide variety of types of membrane bioreactors, which are capable of being constructed and are suitable for carrying out a large number of industrially important processes with effective economical parameters, have been developed. Presumably a more far reaching recourse to the principles of the organisation of processes in biological systems will prove extremely useful for further development of MBr. The creation of more flexible and effective integrated membrane systems may be promoted by the employment of regulatable elements whose properties change under the influence of chemical substances, light, electric or magnetic fields, and also other types of influence. It appears of interest to investigate highly non-equilibrium MBR operating conditions under which spatial-time oscillations arise in the concentrations and fluxes of the components. A far-reaching modulation of properties can be obtained in hybrid systems incorporating particular biological complexes and funcional polymer membranes. In such systems, it is possible to control the mineralistaion of the working volumes and to normalise the supply of substrates as a result of the symport or antiport of the components transferred by the transport proteins. Br, which has extensive functional possibilities and an exceptionally high stability, is a convenient object for use in control elements. In ordered molecular assemblies, Br can play the role of a rapidly acting photoelectric and photoelectrochemical converter. The simplicity and susceptibility to technological formulation of the preparation of Br-containing membrane systems creates preconditions for their practical use. The study of the possible application in hybrid systems, apart from Br, of other energy-dependent biological complexes—the reaction centres of photosynthesising systems, transport ATPases, etc.—is also of undoubted interest.

REFERENCES

1. S.F.Timashev, Uspekhi Khim., 1988, **57**, 876 [Russ.Chem.Rev., 1988(5)].
2. K.Honda, "The 1987 International Congress on Membranes and Membrane Processes (ICOM 87)", Tokyo, 1987, p.6.
3. E.Driolo, "The 1987 International Congress on Membranes and Membrane Processes (ICOM 87)", Tokyo, 1987, p.33.
4. N.Itoh, Y.Shindo, K.Haraya, and T.Hakuta, "The 1987 International Congress on Membranes and Membrane Processes (ICOM 87)", Tokyo, 1987, p.654.
5. T.Kameyama, Ind.Eng.Chem.Fundam., 1981, **20**, 97.
6. V.N.Parmon, Zhur.Vses.Khim.Obshch.im.Mendeleeva, 1986, **31**, 537.
7. Yu.A.Gruzdkov, E.N.Savinov, and V.N.Parmon, "Tezisy Dokladov II Vsesoyuznoi Konferentsii 'Fotokataliticheskoe Preobrazovanie Solnechnoi Energii'" (Abstracts of Reports at the IInd All-Union Conference on 'Photocatalytic Transformation of Solar Energy'), Novosibirsk, 1987, p.65.
8. A.W.-H.Mau, C.-B.Huang, N.Kakuva, A.J.Bard, A.Campion, M.A.Fox, J.M.White, and S.E.Webber, J.Amer.Chem.Soc., 1984, **106**, 6537.
9. S.F.Timashev, "Synthetic Polymeric Membranes" (Edited by B.Sedlacek et al.), Walter de Gruyter, Berlin–New York, 1987, p.437.
10. P.C.Lee and D.Meisel, J.Amer.Chem.Soc., 1980, **102**, 5477.
11. A.M.Mika and K.Lorenz, ICOM 87, 769.
12. L.Campanella, M.Majone, C.Morgia, and A.M.Salvi, ICOM 87, 773.
13. M.H.L.Leiva and V.Gekas, ICOM 87, 461.
14. W.F.Blatt, M.P.Feinberg, H.B.Hopfenberg, and C.A.Savoris, Science, 1965, **150**, 224.
15. W.F.Blatt and S.M.Robinson, Anal.Biochem., 1968, **26**, 151.
16. D.I.C.Wang, T.Sonoyama, and R.I.Mateles, Anal.Biochem., 1968, **26**, 277.
17. T.A.Butterworth, D.I.C.Wang, and A.J.Sinskey, Biotechnol. Bioeng., 1970, **12**, 615.
18. M.Cheryan and M.A.Mehaia, "Membrane Separations in Biotechnology" (Edited by W.C.McGregor), New York–Basel, 1986, p.255.
19. M.C.Porter and A.L.Michaels, Chem.Technol., 1970, **1**, 59.
20. T.Winnicki, ICOM 87, 635.
21. H.Kanayama, T.Tomoyashi, and S.Katayama, ICOM 87, 652.
22. P.R.Rony, J.Amer.Chem.Soc., 1972, **94**, 8247.
23. M.Pizzichini and M.Sperandei, ICOM 87, 212.
24. W.Pronk, A.Knol, and K.Riet, ICOM 87, 637.
25. A.Kilara, K.M.Shahani, and F.M.Wagner, Biotechnol.Bioeng., 1977, **19**, 1703.
26. W.Klagba, J.Braun, and F.Le Goffic, ICOM 87, 639.
27. S.L.Paterson, A.G.Fane, C.J.D.Fell, and P.L.Rogers, ICOM 87, 644.
28. H.N.Chang, B.N.Chung, and J.K.Park, ICOM 87, 658.
29. G.Iorio, E.Drioli, and G.Catapano, ICOM 87, 627.
30. M.Cantarella, L.Cantarella, G.Cirielli, et al., ICOM 87, 646.
31. M.Nakajima, N.Jimbo, K.Nishizawa, et al., ICOM 87, 453.
32. S.A.Leeper, "Membrane Separations in Biotechnology" (Edited by W.C.McGregor), New York–Basel, 1986, p.161.
33. S.D.Varfolomeev and I.V.Berezin, Zhur.Vses.Khim.Obshch. im.Mendeleeva, 1986, **31**, 489.
34. A.G.Erikssen, ICOM 87, 397
35. H.Markl, M.Lecher, and F.Gotz, ICOM 87, 666.
36. E.N.Kondrat'eva and I.N.Gogotov, "Molekulyarnyi Vodorod v Metabolizme Mikroorganizmov" (Molecular Hydrogen in the Metabolism of Micro-organisms), Izd.Nauka, Moscow, 1981, 344 pp.
37. S.D.Varfolomeev and E.S.Pantskhava, "Biotekhnologiya" (Bio-Technology), Izd.Nauka, Moscow, 1984, p.125.
38. I.N.Gogotov, "Biokonversiya Solnechnoi Energii" (Bioconversion of Solar Energy), Pushchino, 1984, p.54.
39. N.A.Zorin, L.T.Serebryakova, I.N.Gogotov, and I.I.Proskuryakov, "Biokonversiya Solnechnoi Energii" (Bioconversion of Solar Energy), Pushchino, 1984, p.167.

40. M.L.Shuler, "Membrane Separations in Biotechnology" (Edited by W.C.McGregor), New York–Basel, 1986, p.303.
41. E.Sada, S.M.Katoh, and M.Terashima, ICOM 87, 635.
42. I.N.Gogotov, L.T.Serebryakova, N.A.Zorin, and K.L.Kovach, "Biokonversiya Solnechnoi Energii" (Bioconversion of Solar Energy), Pushchino, 1984, p.199.
43. V.Ya.Shafirovich and A.E.Shilov, Zhur.Vses.Khm.Obshch.im. Mendeleeva, 1986, **31,** 542.
44. G.Mossa, A.Di Ciulio, L.Aviglano, et al., ICOM 87, 660.
45. T.Kondo, H.Ohshima, A.Kato, and K.Fukuda, ICOM 87, 753.
46. T.M.S.Chang, Science, 1964, **146,** 524.
47. T.M.S.Chang, F.C.MacIntosh, and S.G.Mason, Canad.J.Physiol.Pharmacol., 1966, **44,** 115.
48. Y.Okahata, Acc.Chem.Res., 1986, **19,** 57.
49. G.G.Roberts, Sensors and Actuators, 1983, **4,** 131.
50. H.Fujihira, ICOM 87, 683.
51. U.B.Sleytr and P.Messner, Ann.Rev.Microbiol., 1983, **37,** 311.
52. H.Yamada and M.Fujihira, ICOM 87, 693.
53. W.M.Reichert and J.J.Bruckner, Thin Solid Films, 1987, **152,** 345.
54. S.Nakamoto, J.Kimura, and T.Kuriuama, ICOM 87, 605.
55. M.Gotoh, E.Tamiya, and L.Karube, ICOM 87, 616.
56. G.R.Ivanitskii, "Svetochuvstvitel'nye Biologicheskie Kompleksy i Opticheskaya Registratsiya Informatsii" (Light-Sensitive Biological Complexes and Optical Recording of Information), Pushchino, 1985, p.3.
57. Y.Hirata and H.Fujihira, ICOM 87, 697.
58. H.Shinohara, Y.Iritani, and M.Aizawa, ICOM 87, 620.
59. N.Hakashima, I.Morigushi, K.Nakano, and M.Takagi, ICOM 87, 729.
60. A.Matsuoka, A.Sundon, and Y.Tahekawa, ICOM 87, 614.
61. V.P.Skulachev, "Transformatsiya Energii v Biomembranakh" (Transformation of Energy in Biomembranes), Izd.Nauka, Moscow, 1972, 203 pp.
62. D.Oesterhelt and W.Stoeckenius, Nature, 1971, **233,** 149.
63. L.N.Chekulaeva, M.L.Tsirenina, and B.A.Varver, Ukrain.Biokhim.Zhur., 1980, **52,** 429.
64. Yu.A.Ovchinnikov, N.G.Abdullaev, M.Yu.Feigina, A.V.Kiselev, and N.A.Lobanov, FEBS Lett., 1979, **100,** 219.
65. H.G.Khorana, G.E.Gerber, W.C.Herlihy, C.P.Gray, R.J.Aneregg, K.Nihei, and K.Biemann, Proc.Nat.Acad.Sci. USA, 1979, **76,** 5046.
66. R.Henderson and N.T.Unwin, Nature, 1975, **257,** 28.
67. Yu.A.Ovchinnikov, FEBS Lett, 1982, **148,** 179.
68. R.Henderson Ann.Rev.Biophys.Bioeng., 1977, **6,** 87.
69. D.Oesterhelt, M.Meentzen, and L.Schuman, Eur.J.Biochem., 1973, **40,** 453.
70. S.P.Balashov and F.F.Litvin, "Fotokhimicheskie Prevrashcheniya Bakteriorodopsina" (Photochemical Reactions of Bacteriorhodopsin), Izd.Moskov.Gos.Univ., Moscow, 1985, 164 pp.
71. A.B.Rubin, A.A.Kononenko, V.Z.Pashchenko et al., "Printsipy Regulyatsii i Model'nye Sistemy Pervichnykh Protsessov Fotosinteza. Itogi Nauki i Tekhiniki.Ser.Biofizika" (Principles of the Regulation and Model Systems for Primary Photosynthetic Processes. Advances in Science and Engineering. Biophysics Series), Izd.VINITI, Moscow, 1987, Vol.22, 212 pp.
72. W.Sperling, C.N.Rafferti, K.D.Kohl, and N.A.Dencher, "Energetics and Structure of Halophilic Micro-organisms" (Edited by S.R.Caplan et al.), Elsevier, Amsterdam, 1978, p.321.
73. A.V.Sharkov, A.V.Pakulev, S.V.Chekalin, and Yu.A.Matveetz, Biochim.Biophys.Acta, 1985, **808,** 94.
74. H.-J.Polland, M.A.Franz, W.Zinth, W.Kaiser, E.Koelling and D.Oesterhelt, Biophys.J., 1986, **49,** 651.
75. C.L.Hsien, M.A.Elsayed, M.Nicol, and M.Nagumo, Photochem. Photobiol., 1983, **38,** 83.
76. M.Stockburger, T.Alshuth, and P.Hilderbrand, "The Time Resolved Vibrational Spectroscopy. Proceedings of II International Conference. Emil Warburg Symposium", Berlin, 1985, p.240.
77. K.Gewert and F.Seibert, EMBO J., 1986, **5,** 805.
78. P.Tavan and K.Schulten, Biolphys.J., 1986, **50,** 81.
79. H.Merz and G.Zundel, Biochem.Biophys.Res.Comm., 1981, **101,** 540.
80. M.Engelhard, B.Hess, K.Gerwert, and F.Siebert, Biol.Chem. Hoppe-Seyler, 1987, **368,** 545.
81. L.A.Drachev, A.D.Kaulen, S.A.Ostroumov, and V.P.Skulachev, FEBS Lett., 1974, **39,** 43.
82. L.A.Drachev, A.A.Jasaitis, A.D.Kaulen, A.A.Kondrashin, E.A.Liberman, I.B.Nemecek, S.A.Ostioumov, A.Yu.Semenov and V.P.Skulachev, Nature, 1974, **249,** 321.
83. L.A.Drachev, A.D.Kaulen, L.V.Khitrina, V.P.Skulachev, Eur.J.Biochem., 1981, **117,** 461.
84. G.I.Groma, G.Szabo, and Gy.Varo, Nature, 1984, **308,** 557.
85. H-W.Trissl, Optoelectronik Magazin., 1987, **3,** 105.
86. R.Renthal and J.K.Lanyi, Biochem., 1976, **15,** 2136.
87. E.Racker and W.J.Stoeckenius, Biol.Chem., 1974, **249,** 62.
88. S.-B.Hwang and W.J.Stoeckenius, Membr.Biol., 1977, **33,** 325.
89. M.Happe, R.M.Teather, P.Overath, A.Knobling, and D.Oesterhelt, Biochim.Biophys.Acta, 1977, **465,** 415.
90. L.A.Drachev, A.A.Jasaitis, A.D.Kaulen, A.A.Kondrashin, L.V.Chu, A.Yu.Semenov, I.I.Severina and V.P.Skulachev, J.Biol.Chem., 1976, **251,** 7072.
91. R.Casadio and M.C.Sorgato, Biophys.J., 1984, **45,** Pt.2, 92.
92. G.I.Groma, S.L.Helgerson, P.K.Wolber, D.Beece, Z.Dancshazy, L.Keszthelyi and W.Stockenius, Biophys.J., 1984, **45,** 985.
93. V.M.Mirsky and V.I.Sokolov, Bioelectrochem.Bioenergetics, 1983, **11,** 327.
94. N.Wagner, Eur.J.Biochem., 1987, **165,** 177.
95. L.S.Yaguzhinsky, L.I.Boguslavsky, A.G.Volkov, and A.B.Rakhmaninova, Nature, 1976, **259,** 494.
96. Z.Dancshazy and B.Karvaly, FEBS Lett., 1976, **72,** 136.
97. T.R.Herrman and G.W.Rayfield, Biochim.Biophys.Acta, 1976, **443,** 623.
98. P.Shieth and P.Packer, Biochem.Biophys.Res.Comm., 1976, **71,** 603.
99. L.A.Drachev, A.D.Kaulen, and V.P.Skulachev, Mol.Biol., 1975(11), 1377.
100. R.Toth-Baconadi, S.G.Hristova, L.Keszthelyi, FEBS Lett., 1986, **195,** 164.
101. A.Der, P.Hargittai, and J.Simon, J.Biochem. Biophys.Methods, 1985, **10,** 245.
102. N.N.Vsevolodov, G.R.Ivanitskii, M.S.Soskin, and V.B.Taranenko, Avtometriya, 1986, **2,** 41.
103. M.E.Dumont, J.Tredwhella, D.M.Engelman, F.M.Richards, Membr.Biol., 1985, **88,** 233.
104. T.Shrenkenbach, "Photosynthesis in Relation to Model Systems" (Edited by J.Barber), Elsevier, Amsterdam, 1977.
105. K.Nagy, Biochem.Biophys.Res.Comm., 1978, **85,** 383.
106. Yu.A.Lasarev and E.I.Terpugov, Biochim.Biophys.Acta, 1980, **590,** 324.
107. R.Korenstein and B.Hess, FEBS Lett., 1977, **82,** 1.

108. T.Koyama, R.A.Bogomolni, and W.Stoeckenius Biophys.J., 1985, **48**, 201.
109. S.K.Chamorovskii and A.Ya.Pikulenko, Izv.Akad.Nauk SSSR, Ser.Biol., 1984(2), 294.
110. S.K.Chamorovsky, E.P.Lukashev, A.A.Kononenko, and A.B.Rubin, Biochim. Biophys.Acta, 1983, **725**, 403.
111. Gy.Varo and L.Keszthelyi, Biophys.J., 1983, **43**, 47.
112. P.Hilderbrandt and M.Stockburger, Biochemistry, 1984, **23**, 5539.
113. R.Korenstein and B.Hess, Nature, 1977, **270**, 184.
114. N.N.Vsevolodov and L.N.Chekulaeva, Biofizika, 1978, **23**, 1019.
115. G.W.Rayfield, Photochem.Photobiol., 1986, **43**, 171.
116. N.A.Clark, K.J.Rothschild, D.A.Luippold and B.A.Simon, Biophys.J., 1980, **31**, 85.
117. S.B.Hwang, J.F.Korenbrot, and W.Stoeckenius, Biochim.Biophys.Acta, 1978, **509**, 300.
118. B.Neugebauer, A.E.Blaurock, and D.L.Woveester, FEBS Lett., 1977, **78**, 31.
119. K.A.Fischer, K.Janagimoto, and W.J.Stoeckenius, Cell Biol., 1978, **77**, 611.
120. Gy.Varo, Acta Biol.Acad.Sci.Hung., 1981, **32**, 301.
121. D.Oesterhelt, FEBS Lett., 1976, **64**, 20.
122. H.-W.Trissl and M.Monthal, Nature, 1977, **266**, 655.
123. J.Schildkraut and A.Lewis, Thin Solid Films, 1985, **134**, 13.
124. T.L.Okajima and F.Hong, Biophys.J., 1986, **50**, 901.
125. F.Hong, Biosystems, 1986, **19**, 223.
126. K.Tsuji and E.Neumann, Int.J.Biol.Macromol., 1981, **3**, 231.
127. P.Ormos, L.Reinisch, and L.Keszthelyi, Biochim.Biophys.Acta, 1983, **722**, 471.
128. A.V.Maksimychev, E.P.Lukashev, A.A.Kononenko, et al., Biol. Membrany, 1984, **1**, 294.
129. A.V.Maksimychev, S.K.Chamorovskii, S.F.Timashev, et al., Biol.Membrany, 1984, **1**, 1290.
130. A.A.Kononenko, E.P.Lukashev, A.V.Maximychev, et al., Biochim.Biophys.Acta, 1986, **850**, 162.
131. T.Kinoshita, J.Amer.Chem.Soc., 1986, **608**, 6399.
132. T.Kinoshita, Colloid.Polym.Sci., 1983, **261**, 933.
133. K.Nishiyama and M.Fujihura, ICOM 87, 707.
134. M.Maeda, D.-W.Chung, and S.Inoue, ICOM 87, 255.
135. A.J.Takizawa, Polym.Sci.Polym.Chem.Ed., 1986, **24**, 665.
136. Y.Okahata and G.En-na, ICOM 87, 656.
137. M.Aizawa, S.Yabuki, H.Shinohara, and T.Chiba, ICOM 87, 631.
138. T.Katsura, ICOM 87, 234.
139. H.G.Khorana, "Microbial Energy Transduction: Genetic Structure and Function of Membrane Proteins. Cold Spring Harbour Conference, 1986", New York, 1986, p.141.

Karpov Physicochemical
Institute, Moscow
Biological Faculty,
Moscow State University