lyzed by the enzyme. To date, there are many substrates that have been studied by the use of oxidoreductases, and the majority of the enzyme biosensors have been designed specifically for the determination of a large number of "cardiac" enzymes in blood.

Tissue materials from plant and mammalian sources have been successfully employed for the construction of biosensors as well (1). This class of biocatalytic materials simply maintains the enzyme of interest in its natural environment (e.g., lipid bilayer), which results in a considerable stabilization of the desired enzymatic activity.

The microbial sensors are composed of immobilized microorganisms and an electrochemical device and are suitable for the on-line control of biochemical processes. These sensors involve the assimilation of organic compounds by the microorganisms, change in respiration activity, or production of electrooactive metabolites. These changes have been monitored directly by an electrochemical device.

The sensitivity of electrical measurements developed for the electrochemical biosensors, coupled with the specificity of antigen–antibody reactions, provides a useful tool for immunology. However, more recently, the optical sensors for immunoassays have been receiving considerable attention in research laboratories and also *in vivo* applications. Among different types of optical biosensors, two types appear to be especially promising. One is based on a surface plasmon resonance phenomenon, and the second one is a fluorescence capillary fill device. Surface plasmon resonance in a thin metal film deposited on a wave guide can be induced by an electromagnetic wave generated when the light is reflected within the wave guide, and it is highly sensitive to variations in the refractive index of the immediate surrounding medium. This phenomenon is monitored by a reduction in the intensity of the reflected light (2).

Biosensors: Diversity and Development

Generally, the biological components used in a biosensor construction can be divided into two categories: (a) those where the primary sensing event results from catalysis (such as whole microorganisms, tissue slices, organelles, and enzymes) and (b) those which depend on an essentially long-term binding of the target molecule (i.e., cell receptors, nucleic acids, and antibodies). The essential element, however, in making a successful biosensor is to provide a suitable immobilization procedure for biological compounds. Five main approaches to enzyme immobilization have been employed: (1) physical adsorption at a solid surface, (2) entrapment in polymeric gel or within microcapsules, (3) cross-linking by means of bifunctional reagents, often in combination with (1) and (2), (4) covalent binding to a reactive insoluble support, and (5) embedding in a lipid bilayer. This last-mentioned approach is unique in that embedding means that the compound(s) (membrane modifiers such as electron acceptors, donors, mediators, polypeptides, proteins, etc.) of interest in the lipid bilayer is relatively free to adapt to its surroundings. The functions of biomembranes are mediated by specific modifiers, which assume their active conformations *only* in the lipid bilayer environment. Furthermore, the presence of the lipid bilayer greatly reduces the background noise (interference). It also effectively excludes hydrophilic electroactive compounds from reaching the detecting surface that may cause undesired reac-

INTELLIGENT BIOSENSORS

OVERVIEW OF A BIOSENSOR

The interaction of biophysics with cell physiology and molecular biology has given rise to an exciting area of research termed membrane biophysics, which integrates up-to-date findings on molecules and processes involved in inter- and intracellular recognition and communication. Knowledge of the ideas and findings resulting from such interdisciplinary research are now being used for practical applications in analytical chemistry, immunology, photobiology, chemical/biological sensors and transducers, and molecular electronics.

What is a Biosensor?

A biosensor is an analytical device incorporating biological and chemical sensing elements either intimately connected to, or integrated with, a suitable transducer, enabling the conversion of concentrations of a specific species into digital electronic signals. A majority of the biosensors developed thus far have incorporated an enzyme as a biological recognition component. For example, all types of enzyme sensors are based on the classic idea which measures glucose by detecting the reduction in oxygen when the oxidation of glucose is cata-

tions. From the specificity, selectivity, speed of response, and design points of view, a supported planar bilayer lipid membrane (s-BLM) is an ideal natural environment for embedding a host of materials of interest. Hence, the s-BLM system offers a wider opportunity for the biosensor development (3). In the following we shall be mainly concerned with the electrochemical biosensors based on supported BLMs using electrical detection methods.

In practice, researchers continue to search for ideal combinations of biocatalysts–enzymes, antibodies–antigens, bacteria, plant slices isolated receptors, and even whole cells. In this context, genetic engineering technology will have a role in improving the biological component of enzyme-based and whole cell biosensors. Materials science and chemical engineering are instrumental in finding solutions for such problems as suitable immobilization procedures, transducers, and the effective coupling of the biological component to the transducer and the subsequent amplification system. The main types of transducers used in biosensor construction are listed in Table 1. Although in recent years a variety of different biological components have been used (1), it is by no means certain that all possible combinations of sensing elements and transducers have been explored.

The main challenge to the scientist in biosensor technology is to find an effective coupling of the biological component to the transducer. This is particularly important in the development of amperometric biosensors, since conventional "clean" metal electrodes are generally very poor voltammetric electrodes for the direct oxidation or reduction of biological components. A host of approaches to the modification of electrodes have been developed and investigated. All those methods, however, can be divided into two groups: (a) the modification of the electrode surface by depositing a monolayer, which is based upon either the adsorption of species at the electrode surface or a covalent attachment of redox mediators to the electrode, and (b) the modification by a multilayer, which is most frequently achieved by the use of polymeric modification of the electrode or sometimes by a vapor-phase deposition. The use of mediators in conjunction with oxidoreductases is not a recent innovation. Molecules such as quinones, organic and inorganic ions, and redox dyes have all been used. More recently, a novel electrode based on highly conducting metals and polymeric electron transfer mediators has been used. A practical mediator, however, should fulfill certain criteria. One of the most successful classes of electron mediators have been highly conjugated compounds such as ferrocene (biscyclopentadienyl iron), tetracyanoquinodimethane (TCNQ), tetrathiafulvalene (TTF), and their derivatives. However, very recently, avidin biotin coupling arose as a promising method for preparing enzyme-based biosensors (1,4).

Table 1. Types of Transducers and Measurements Used in Biosensor Technology

Transducers	Measurement
Oxygen electrode	Electrochemical
Ion-selective electrode	Potentiometry
Modified metal electrode	Amperometry
Field-effect transistor	Conductimetry
Conductometry	Impedometry
Spectrophotometry	Photometric
Laser light scattering	
Optical fibers combine with absorption and fluorescence	
Surface plasmon resonance	
Thermistors	Thermometric
Piezoelectric crystal	Acoustic
Surface acoustic wave device	

Recent success in the interdisciplinary research in biology combined with electronics has led to exciting new developments based on enzymology and transducer techniques. They are known as enzyme electrodes, enzyme thermistor, CHEMFET/ENFET devices, and immunosensor or enzyme transistor. Collectively, they are called "biosensors or biochips" (5). A common feature of all these devices is a close connection between the enzyme and the transducing system, which is used to follow the enzymatic reaction. The essential principle of the devices, broadly speaking, is predicated on the ligand–receptor contact interactions (6). Application of such developments in the fields of medicine, pharmaceuticals, biochemistry, environmental settings, robotics, and the food industry are obvious. For example, enzyme thermistors make use of the heat which is liberated during an enzymatic reaction. Their usual sensitivity is around 0.01°C. A recent modification of enzyme thermistor is the "TELISA" electrode, which achieves a sensitivity of about 10^{-13} M using an immunoabsorbent. It is expected that this measuring technique will find a broad application in continuous measurements of the release of hormones and/or antigens–antibodies in blood circulation. The quick attainment of a new steady state in the reaction occurring at an enzyme electrode after a random perturbation makes the latter ideally suited to monitor an industrial process (e.g., the production of antibodies). Classic calorimetric methods require much more time than an enzyme thermistor assay to perform a quantitative analysis. Two other interesting developments are ellipsometry and piezoelectric crystals. In ellipsometry, a close connection between the enzyme and the transducing device is not required. The method relies on the change in the angle of polarization of incident light which is reflected by a layer of biomolecules bound to a solid surface. A change in the thickness and conformation of this layer, under the influence of other macromolecules interaction with the layer, can be easily monitored. This principle is now used in the fermentation industry. Piezoelectric crystals can be used in the analysis of the traces of certain compounds, mainly anesthetics. The frequency of the crystal depends strongly on the absence or presence of adsorbed molecules on the surface of the crystal (7). The selectivity of crystals toward a given compound may be increased by a coating process (e.g., with hydrophobic substances such as oils and fats).

Another exciting new research area is the combination of semiconductor technology with enzymes and other biological macromolecules. Here, mostly field-effect transistors (FETs) are used. If sensitivity of a FET toward certain chemicals or ions can be achieved, the prototype of an "ISFET" is born. A common feature of all these devices is the use of metal oxide semiconductor (MOS) structure. In combination with a thin layer of palladium, a high sensitivity toward gaseous hydrogen can be achieved. In this case, the membrane separates the gaseous from the liquid phase. Addition of traces of certain metals (e.g., Ir) to the Pd-MOS device also increased its sensitivity toward ammonia. It has been shown that such a

device is capable of monitoring reliably the production of hydrogen by microorganisms (e.g., *Clostridium acetobutylicium*).

Lipid-Bilayer-Based Biosensors

The basic unit of all living organisms is the cell. Each cell is bound by a limiting plasma membrane, the fundamental structure of which is a lipid bilayer modified with proteins and carbohydrates. This is so because of the unique property of the lipid molecule: One end of the molecule is hydrophilic with a strong affinity toward a medium of high dielectric constant, while the other end of the molecule is hydrophobic, which is sequestered away from the aqueous solution. We now know that the remarkable stability of a lipid bilayer is due to the combination of hydrophilic and hydrophobic forces, which makes the lipid bilayer a thermodynamically favored structure (8).

The living cells undertake intercellular communication, which take place across as well as between cell membranes. The intercellular communication is mediated by the signal-transducing system, which is undertaken by various sensitive biomolecules (or sensor molecules) such as enzymes, ion channels, receptors, and carriers. These sensors *in vivo* serve as communicators to send, receive, transfer, and decode signal to adapt the cell to the changed environment. This ability of living cells is so-called biosensitivity. These signals are generally electrical and chemical in nature, predicated upon the presence of membrane. From the viewpoint of membrane biophysics and physiology, biological membranes are essentially the supporting matrix of the nature's sensors and devices (3,4), and the cell membrane plays a crucial role in signal transduction, energy conversion, and information processing. This is due to the fact that most physiological activities involve some kind of lipid bilayer-based ligand–receptor contact interactions. Outstanding examples among these are ion sensing, antigen–antibody binding, light conversion and detection, and gated channels, to name a few. For example, the thylakoid membrane of green plants functions as an energy transducer converting sunlight into electrical/chemical energy, the photoreceptor membrane of a rod's outer segment detects photons as the initial step in visual perception, and the plasma membrane of cells and organelles possess the ability for ion sensing—for instance, differentiating Na^+ and K^+ with a great specificity. Furthermore, the plasma membrane provides sites for a host of ligand–receptor contact interactions such as antigen–antibody formation (2–4).

These outstanding characteristics have contributed to the discoveries of two artificial bilayer lipid membrane systems—namely, planar BLMs (9) and spherical liposomes (10)—in 1960s. The seminal work on the self-assembly of planar, bilayer or "black" lipid membranes (BLMs) was carried out by Rudin and his associates in 1959 to 1963. The idea started while one of the authors was reading a paperback edition of *Soap Bubbles* by C. V. Boys (11). These early researchers realized that a soap film in air in its final stage of thinning has a structure which may be depicted as two monolayers sandwiching an aqueous surfactant solution. Rudin and co-workers showed that an under water "soap bubble" (i.e., a BLM formed from brain extracts) was self-sealing and resistant to puncture and had many physical and chemical properties similar to those of biomembranes (2,9). Since then, techniques have been developed to incorporate a wide variety of compounds into BLMs to endow them with desired properties. As a result of the efforts of many investigators (12–15), biologically relevant phenomena such as ion selectivity, excitability, antibody–antigen reactions, active ion transport, and photoelectric effects have all been demonstrated.

In recent years, numerous attempts have been made to exploit the BLM system's potential in practical applications in sensor (1). Advances in microelectronics and interest in ultrathin organic films, including the BLM, especially the newly developed self-assembled bilayer lipid membrane (s-BLM) on a nascent metallic surface (16), have resulted in a unique fusion of ideas towards the development of intelligent biosensor and transducer. Furthermore, recent trends in interdisciplinary studies in chemistry, electronics, and biology have led to a new field of scientific–technological endeavor which is a part of a more general approach toward the development of a new, post-semiconductor electronic technology, namely, molecular electronics with a long-term goal of molecular computers (17).

SELF-ASSEMBLED LIPID BILAYER: TECHNOLOGY AND MEASUREMENT

Techniques of Planar BLMs and s-BLMs

The history of the BLM system and its development as a model for biomembranes has been recounted elsewhere (9). It should be mentioned that there is one major difference between Langmuir–Blodgett (L–B) film layers on rigid substrates and the BLMs. Apart from its bimolecular thickness, a BLM is a liquid-like, dynamic structure in a metastable state from a self-assembling point of view; it is envisioned that for long-term stability, a BLM separating two aqueous solutions requires the presence of a Plateau–Gibbs border. For biosensor development, it is our opinion that a fluid bilayer is of crucial importance. The aim of this section is to describe in sufficient detail how to set up a simple BLM system using the self-assembling techniques (see Table 2).

Conventional Planar BLMs. An experimental BLM, composed of either a mixture of 1% phosphatidylcholine and cholesterol in *n*-decane or oxidized cholesterol (ox. chol.) in *n*-octane, is formed in an aperture (diameter from 0.1 mm to 2 mm depending on the experimental requirement) punched in the side of a 10 ml Teflon cup which sits in an outer chamber of 20 ml volume. Before the introduction of the lipid solution, both chambers are filled with 0.1 M KCl. The lipid solution is then injected over the orifice of the Teflon cup in the bathing solution with a micro-syringe. The lipid solution should cover the opening over the entire orifice. One should see the light reflected from the thinning lipid film with iridescent color patterns. These colors gradually disappear as the membrane thins, and the membrane appears "black" when a BLM is finally formed. That is because such an ultrathin planar BLM possesses two interfaces and its thickness is less than 7 nm compared to the wavelength of visible light, and the membrane appears "black" because the light reflected from the front interface undergoes a half wavelength phase shift and interferes destructively with the light reflected from the back interface, which experiences essentially no phase shift. The BLM formation is monitored by the increase in membrane capacitance via a pair of Ag/AgCl electrodes. Electrical pa-

Table 2. Self-Assembling Systems Containing Amphiphilic Molecules

System	Literature Source
1. Soap films	R. Hooke, in *The History of the Royal Society of London,* **3:** 29, 1672.
2. Monolayers	I. Langmuir, *J. Am. Chem. Soc.,* **39:** 1848, 1917.
3. Langmuir–Blodgett multilayers	K. B. Blodgett and I. Langmuir, *Phys. Rev.,* **51:** 964, 1937.
4. Planar bilayer lipid membranes (BLMs)	P. Mueller et al., *Nature,* **194:** 979, 1962.
Liposomes (lipid microvesicles)	A. D. Bangham, *BioEssays,* **17**(12): 1081, 1995.
5. Nucleopore supported BLMs	J. D. Mountz and H. T. Tien, *Photochem. Photobiol.,* **28:** 395–400, 1978.
6. Gold supported monolayers	(a) L. Taniguchi et al., *J. Electroanal. Chem.,* **140:** 187, 1982.
	(b) R. G. Nuzzo and D. L. Allara, *J. Am. Chem. Soc.,* **105:** 4481, 1983.
	(c) L. Netzer and J. Sagiv, *J. Am. Chem. Soc.,* **105:** 674; 1983.
7. Metal supported BLMs (s-BLMs)	H. T. Tien and Z. Salamon, *Bioelectrochem. Bioenerg.,* **22:** 211, 1989.
8. Salt-bridge supported BLMs (sb-BLMs)	(a) H.-P. Yuan et al., *Mater. Sci. Eng. C,* **4:** 35–38, 1996.
	(b) X.-D. Lu et al., *Bioelectrochem. Bioenergetics,* **39:** 285–289, 1996.

rameters of the BLM are measured with a high gain electrometer and a picoammeter. A number of simple setups for the BLM study have been published (9,18), and several comprehensive reviews on the subject are available (12–15).

Supported Bilayer Lipid Membranes (s-BLMs): The New BLM System. Since BLM's inception in 1960, many modifications have been made to the original BLM technique, with one notable exception to be described below, but the essential principle has remained the same. A BLM formed in the conventional manner (as described in the last section) is an extremely fragile structure with a limited lifetime. For long-term basic studies as well as for technological applications, the common concern has been the mechanical stability of the BLM. Although a number of improvements have been made to prolong the lifetime of the BLM, they rarely last longer than a few hours. For protracted studies and practical applications such as in biosensors and molecular electronic devices development, a long-lived BLM is a prerequisite. For this reason, it is both desirable and imperative that a method be found so that long-lasting BLMs can be generated. In 1989, a simple and novel method was reported for the formation of self-assembled BLMs on solid substrates, which possessed the desired dynamic properties and the requisite mechanical stability (6,16).

The principle and potentialities of the original method of the s-BLM probe construction are as follows. The two-step procedure consists of the adsorption of lipids from a BLM-forming solution on a nascent metal surface followed by the immersion of the lipid-coated tip into an aqueous solution for a BLM to self-assemble (16). This method is very simple, and it rivals the much more complicated and widely used Langmuir–Blodgett (L-B) film technique for certain biosensor fabrication (Table 2). Experimentally, s-BLMs on solid support have been formed by a number of methods, and the two-consecutive steps technique is carried out in the following manner: (1) Place a Teflon-coated metal wire [e.g., platinum (Pt) or stainless steel (ss)] and cut the tip of the wire with a sharp blade, while it is in contact with a BLM-forming solution. (2) Immerse the lipid layer that has adsorbed onto the metal wire tip into an aqueous solution for 5 min to 10 min for a BLM to self-assemble [see Fig. 1(a)]. For best cutting of the metal wire a miniature guillotine has been used, in which the sharp knife is moved vertically onto the wire placed on a flat surface and immersed in a lipid solution (16). Typically we used either an 1% glycerol dioleate in squalene or 5% phospholipid (PC or lecithin) in *n*-decane. For the probe construction we used Teflon-coated ss wire (0.05 cm diameter). Assuming the molecular area of PC to be 50 $Å^2$, about 2×10^{14} molecules are needed to cover 1 cm^2 of metal surface with a BLM.

Alternatively, an s-BLM can also be formed at the end of a Teflon tubing filled with a hydrogel in KCl as a salt bridge used in electrochemistry. The formation procedure consists of three steps. In the first step, a chlorided Ag wire (Ag/AgCl) is inserted into the Teflon tubing which has been previously filled with a mixture of agar (or agarose) and KCl solution saturated with AgCl (0.3 g agar in 15 ml of 3 M KCl). The AgCl electrode and the filled Teflon tubing are glued together with wax at the point of insertion. In this way an Ag/AgCl–Teflon salt bridge is constructed. In the second step, the tip of the other end of the Teflon salt bridge is cut *in situ* while immersed in a BLM-forming solution with a scalpel. In the third and the last step, the Ag/AgCl–Teflon salt bridge with the freshly lipid solution coated tip is immersed in 0.1 M KCl solution of the cell chamber. The second step described above may be carried out in air and then the freshly cut end of the salt bridge is immediately immersed in the lipid solution for a few minutes. In either case, the cell chamber fills with an appropriate aqueous solution (e.g., 0.1 M KCl) containing an Ag/AgCl reference electrode and an Ag/AgCl–Teflon salt bridge with a self-assembled BLM at its end. The lead wires of the two electrodes are connected to the measuring instrumentation (see Fig. 2).

The precise arrangement and degree of ordering of the lipid molecules in the final structure is not known for certain. However, it seems highly probable that the bilayer nature of the assembly is a consequence of the thermodynamics of free-energy changes at the metal–lipid and lipid–aqueous solution interfaces.

Instrumentation. A number of methods that study the properties of BLMs such as optical, electrical, mechanical, transport, and permeability have been developed over the years (2,12,13,19). Of these methods, we shall describe only the electrical methods. In the last decade, many new electrochemical methods have been developed and applied to membrane research (20). Among these, the cyclic voltammetry (CV) turns out to be a very powerful method. The basics of CV consist of cycling the potential of a working electrode in an unstirred solution and measuring the resulting current. The potential of the working electrode is controlled relative to a reference electrode which is provided by a triangular poten-

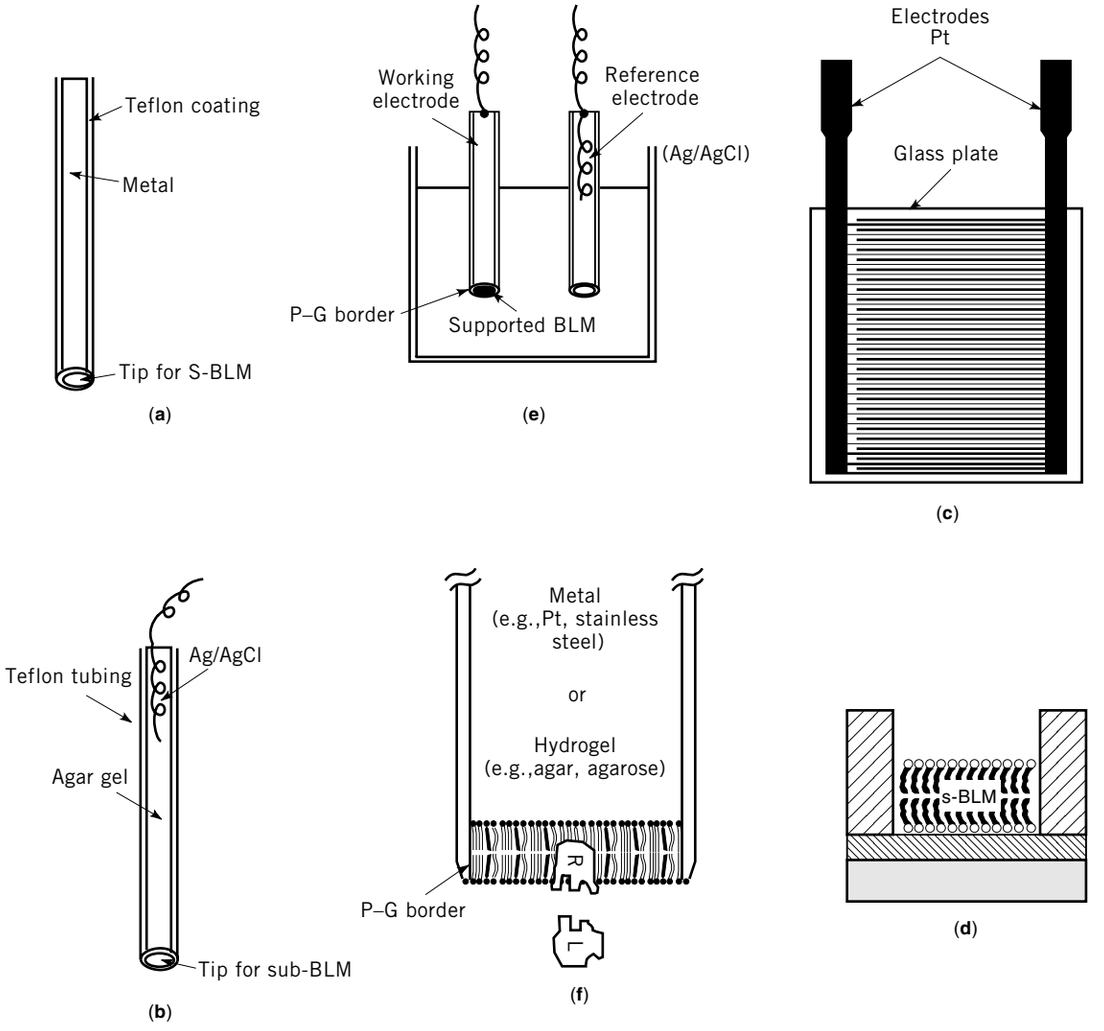

Figure 1. Schematic representations of (a) a metal-supported s-BLM probe, (b) a hydrogel-supported sb-BLM probe (35,50), (c) an interdigitated structure used for supporting a s-BLM (23), (d) a microsystem on a glass substrate for supporting s-BLMs (23), (e) a cell for electrochemical measurements (32), and (f) an enlarged view of the supported BLM illustrating the receptor (R)–ligand (L) interaction. P–G denotes Plateau–Gibbs border (4).

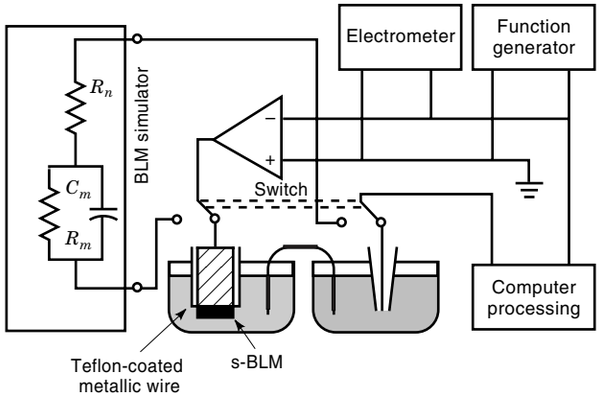

Figure 2. Block diagram of the setup for cyclic voltammetry on the s-BLM system.

tial waveform generator. The instrumentation used with BLMs can be much simpler than that used in the conventional CV, owing to the fact that the high resistance of BLMs can be studied with a two-electrode setup. Thus, a picoammeter together with a voltage waveform generator is all that is required. If a computer or an X–Y displaying device is available, the current–voltage (I–V) curves may be obtained, which are known as voltammograms. From such voltammograms, information about thermodynamic and kinetic parameters of the BLM system may be obtained, thereby providing insights into the mechanism of the membrane process under investigation.

A block diagram of the setup for obtaining cyclic voltammograms of the s-BLM system is shown in Fig. 2. The supporting metallic wire serves as a working electrode in a one-cell chamber [Fig. 1(e)]. The reference electrode, usually a chlorided Ag wire (Ag/AgCl) electrode, is dipped in the 0.1 M KCl solution placed in another chamber, and a salt bridge spans over the two chambers. For a two-electrode system as

is usually used in the measurement, the newly cut tip of the metallic wire, coated with adsorbed lipids, acts as the working electrode. The current through the s-BLM is measured in the auxiliary electrode (made of a coil of Pt wire) during the CV scanning. The setup is housed in a Faraday cage to minimize interference by the external noise and electrical transients. Despite shielding, the external noise may still be picked up by the switch box; therefore, for the critical measurements the switch box should be incorporated within the same Faraday shield as the cell. All cables used are shielded and the shields are grounded.

On-Line Measurement of BLMs from Voltammograms

Parameters Determination of Planar BLMs. The I–V response of an unmodified BLM has a form of parallelogram under the triangle sweeping wave. Figure 3(a) (top) is a typical voltammogram of conventional planar BLM system. A typical equivalent circuit of the planar BLM system is represented as illustrated in Fig. 3(a) (bottom) by a membrane resistance R_m in parallel with a membrane capacitance C_m. The parameters such as the capacitance, resistance, and membrane potential can be determined easily by analyzing these voltammograms. The triangular sweeping wave in the range of $\pm V_0$ with the scan rate A ($\mathrm{mV \cdot s^{-1}}$) is the input from the circuit. The current in nanoamperes or picoamperes is measured. There are two components in the current through the membrane, namely the charging current i_c and resistance current i_r. The former is determined by the capacitance as follows:

$$i_c = C_m \frac{dV}{dt} = C_m A \tag{1}$$

It can be shown that the capacitance current i_c through the membrane capacitance is a constant. From Ohm's law, the latter component i_r of the membrane current is caused by the membrane resistance, that is,

$$i_r = \frac{V}{R_m} \tag{2}$$

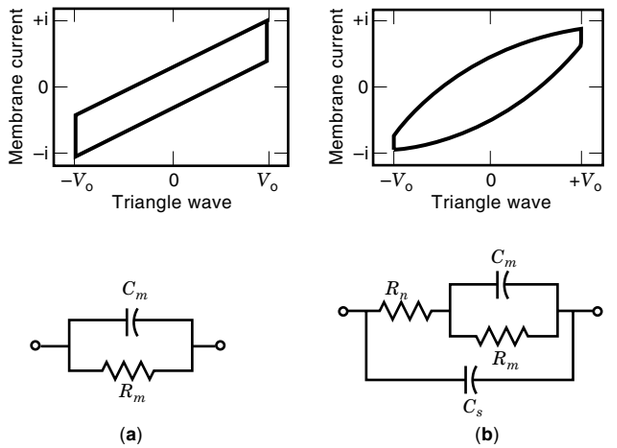

Figure 3. Cyclic voltammograms of BLMs and relevant equivalent circuits: (a) Voltammograms of the planar BLM system (top) and equivalent circuit (bottom). (b) voltammograms of the s-BLM (top) and the improved equivalent circuit (bottom).

So the net current passing through the BLM can be expressed as

$$i = i_r + i_c = \frac{V}{R_m} + C_m A \tag{3}$$

Equation 3 shows that the current through the resistor increases with increasing scan voltage. In the case of the constant scan rate A, with fixed values of C_m and R_m, the current i has a linear relationship with the sweeping potential V. Thus the slope reflects the value of R_m, whereas C_m can be determined by measuring i_c according to the graph of the I–V response. i_c will jump to its negative values suddenly ($-i_c$) only at such points where the sweeping wave reaches its maximum and begins to reverse. The jump distance $2h$ equals $2i_c$, and thus C_m will be calculated by merely measuring h.

Parameters Determination of Supported BLMs. When the CV technique is applied to the solid-supported BLM, it has been found that the shape of I–V curve is quite different from that of a planar BLM (parallelogram). The typical voltammogram of an unmodified s-BLM shown in Fig. 3(b) (top) obviously has a different shape when compared with parallelograms obtained for the planar BLMs in Fig. 3(a) (top). The difference between them indicates that the equivalent circuit as well as the corresponding methods for the measurement of conventional BLM properties are no longer rigorously applicable to the s-BLM system. Measurement errors will have a great impact on the accuracy of the parameter determination unless the circuit is improved. Therefore it is necessary to improve the original equivalent circuit and to establish proper parameters relations using a set of differential equations so that the accurate determination of electrical properties of the s-BLM is assured (20,21).

A typical s-BLM system consists of a supported BLM as the working electrode, an electrolyte, and a reference electrode, which may be represented by a number of suitably connected resistors and capacitors such as R_m and C_m. According to the I–V response recorded on the real s-BLM in Fig. 3(b), the improved equivalent circuit for the s-BLM is developed in Fig. 3(b) (bottom), where the nonmembrane resistance R_n is introduced in series with the original circuit, and the parallel C_s is the distributing capacitance for the entire circuit. The membrane is characterized by a high resistance and a high capacitance. Their measured accuracies are far more affected by the proposed nonmembrane resistance R_n, whose composition and effect on the measurement error will be discussed in the following section.

From simulation we have obtained similar I–V responses by CV with the BLM simulator which contains relevant electrical circuit (see Fig. 2). The effect of R_n is achieved by a set of I–V responses generated by a BLM simulator with and without R_n as shown in Fig. 4, where the regular development of the CV waveforms are shown with varying values of R_m, C_m, R_n, and C_s. Figure 4(a) is a set of voltammograms in which C_m is varied while holding R_m constant and R_n at 0 Ω. From cycle 1 to 3, the intercept with the i axis rises gradually in correspondence with the increasing C_m. In Fig. 4(b) the slope drops down from curve 1 to curve 3 as R_m increases while keeping C_m constant.

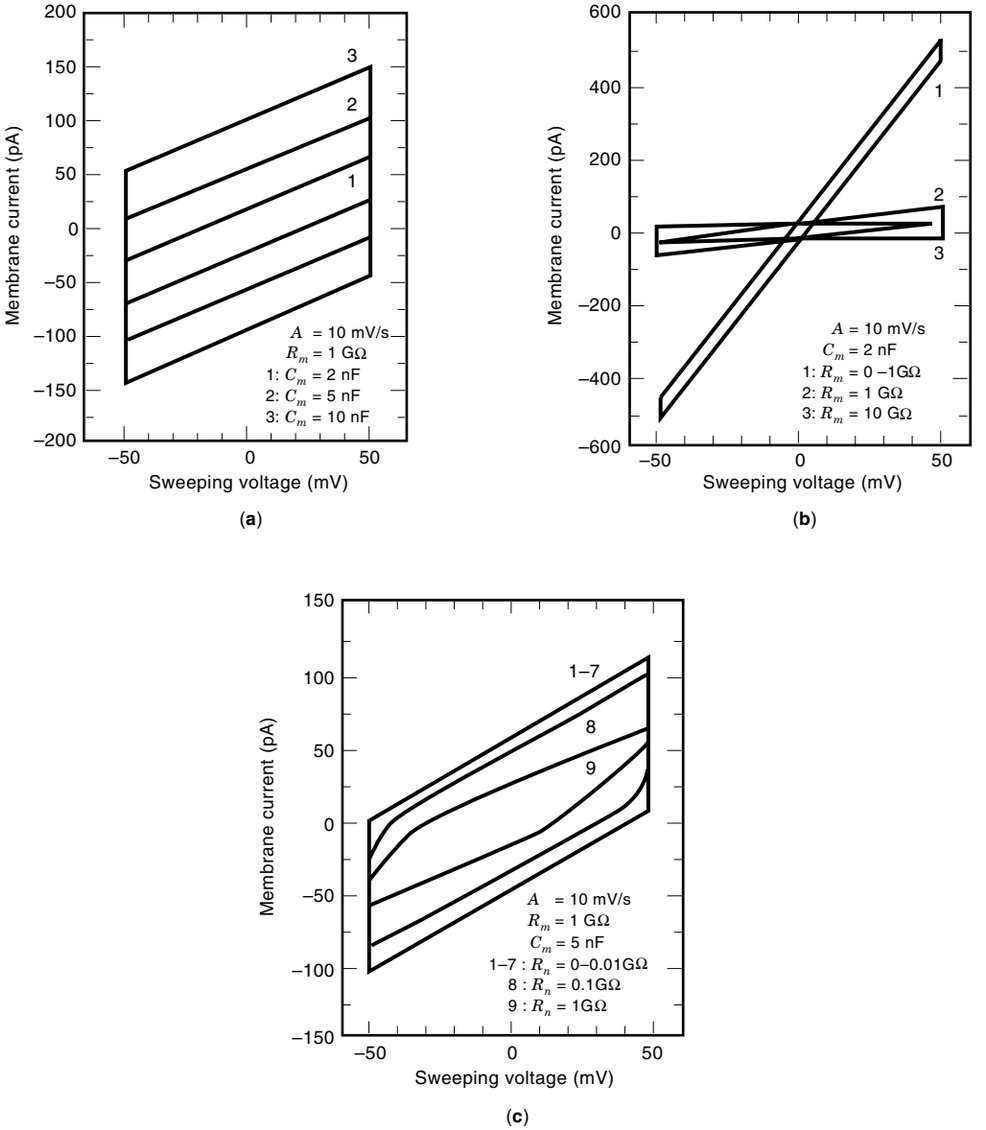

Figure 4. Voltammograms of the BLM simulator: (a) Voltammograms with different values of C_m ($C_m^1 < C_m^2 < C_m^3$) while remaining R_n constant; (b) voltammograms with different R_m($R_m^1 < R_m^2 < R_m^3$), while remaining C_m constant; (c) affection of R_n on voltammograms (cycles 1–7, $R_n \approx$ 0–0.01 GΩ; cycle 8, $R_n \approx 0.1$ GΩ; cycle 9, $R_n \approx 1$ GΩ).

Figure 4(c) is based on the improved equivalent circuit [see Fig. 3(b)], where both R_n and C_s are involved in the consideration. A detailed observation of the effect of R_n on the shape of the I–V cycle is carried out . In our experiment, R_n varies in a wide range from 10^0 to 10^9 Ω, while the other membrane properties are simulated by holding R_m at 10^9 Ω, C_m at 5 nF, both of which are still within the range of biomembrane. Curves 1 to 7 display the similarities of the I–V cycles which have the shape of parallelograms even if R_n reaches the value of 10^7 Ω. However, when R_n increases to 10^8 Ω ($\approx$0.1 R_m), it begins to be characterized by charging current (curve 8). The intercept with the current axis is also somewhat decreased. The most important feature is that, when R_n reaches the same order as R_m ($\approx R_m$), the voltammogram (curve 9) does not display the shape of a parallelogram any longer, and it is very similar to that observed for the s-BLM system [see Fig. 3(b)]. The intercept with the i axis drops down sharply. The slope decreases during the potential sweeping, and it is much lower than the slope in the case of the BLM simulator without R_n or with a low value of R_n (cycles 1 to 7). In this last case, the intercept can no longer reflect the membrane capacitance, and it is the same as the slope of membrane resistance.

Therefore, the measurement error would be greatly increased if one still considers the intercept with the i-axis as the membrane capacitance and the slope as the resistance. What causes the large value of R_n is still being discussed. The previous report using the LAPS technique has studied the system by considering the solution impedance effect (18). However, the value is not so high that it approaches R_m.

Based on our previous research the nonmembrane resistance was not found so high in conventional planar BLM, and the technique for forming self-organized s-BLMs is based conceptually on interactions between a nascent metallic surface and amphipathic lipid molecules. It is supposed that an additional factor is owing to the presence of the metal–lipid interface (interfacial resistance) of R_n. Other important details in R_n include the electrode, electrolyte and solution impedance, and any other components which greatly effect the measurement accuracy.

Here, we propose a new method for determining R_m, C_m, C_s, and R_n accurately from the on-line s-BLM cyclic voltammogram (21). To make clear the effect of R_n on the I–V response, the quantitative relationship of the membrane current with the sweep wave potential has been derived rigorously from the solution of coupled equations based on the equivalent circuit shown in Fig. 3(b). The set of differential equations is as follows:

$$\begin{aligned} i &= i_r + i_c + i_s \\ i_r R_m &= \frac{1}{C_m}\int_0^t i_c\,dt \\ V &= (i_r + i_c)R_n + \frac{1}{C_m}\int_0^t i_c\,dt \\ i_s &= C_s A \end{aligned} \tag{4}$$

where i is the total current recorded, i_s is defined as the current through C_s, and all other parameters are defined above.

The final (definite) solution $i = f(V)$ is presented, where R_n and C_s have been included:

$$i = \frac{AR_mC_m}{R_m + R_n}\left[\frac{V}{AR_mC_m} + \frac{R_m}{R_m + R_n} - \frac{2R_m}{R_m + R_m}\exp\left(-\frac{(R_m + R_n)(V + V_0)}{AR_nR_mC_m}\right)\right] + C_s \cdot A \tag{5}$$

The initial condition for Eq. (5) is

$$V_{t=0} = -V_0 + A \cdot t_{t=0} = -V_0 \tag{6}$$

During the half-cycle sweep from $-V_0$ to $+V_0$, we can see from Eq. (5) that there are three components included in membrane current: (a) the time-linearly dependent term, which is the resistance current obeying Ohm's law; (b) the time-exponentially dependent term in which R_n, R_m and C_m should be taken into account; and (c) the constant term resulting from the distributing capacitance C_s for the entire circuit.

The traditional way for the parameter determination is to vary each of the parameter values while holding all other parameters constant and to select a fitting curve with a minimal deviation. However, the complexity of the calculation is the major impediment in finding a group of effective suitable values to fit the calculated curve. Despite the aid of a computer, the calculation still requires much more time, which cannot be applied in the on-line parameter determination. So it is necessary to derive a set of accurate expressions from the original solution of Eq. (5), by which one-step calculation for determination of R_m, C_m, R_n, and C_s can be conducted.

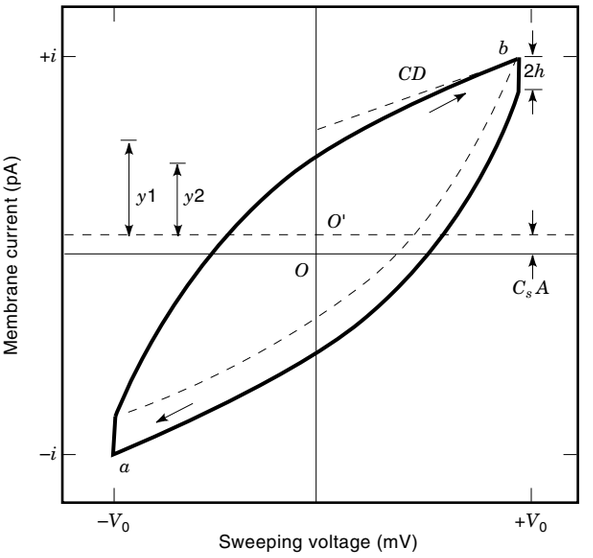

Figure 5. Method for on-line calculation of membrane parameters from s-BLM voltammogram. Four parameters are needed to be acquired from the on-line voltammogram: (1) $1/D$, the slope of the tangent line CD at the half-cycle terminal b ($+V_0$); (2) y_1, the intercept of tangent line CD with the current i-axis; (3) y_2, the intercept of the voltammogram with current i-axis; (4) $2h$, the transient current change at the time where the sweeping voltage reaches the maximum, and begins to reverse. Following Eq. (12) in the text, the membrane parameters can be calculated right away after a one-cycle voltammogram is acquired.

Figure 5 presents this method to determine R_m, C_m, R_n, and C_s. First of all, the constant term C_sA in Eq. (5) will be negative at the very moment when the sweep potential reaches its maximum and begins to reverse. So, the height $2h$ in Fig. 5 corresponds to twice the value of the distribution capacitance C_s multiplied with A. So from on-line computer acquire and parameters determination, the height $2h$ can be read out, and C_s is determined by

$$C_s = h/A \tag{7}$$

The differentiation of Eq. (5) is given by

$$\frac{di}{dV} = \frac{1}{R_m + R_n} + \frac{2R_m}{R_n(R_m + R_n)}\exp\left(-\frac{(R_m + R_n)(V + V_0)}{AR_nR_mC_m}\right) \tag{8}$$

The potential of the triangular sweep wave moves from $-V_0$ to $+V_0$ (from a to b in Fig. 5). At the half-cycle terminal b, the exponential term can be rationally omitted and the slope of the tangent line CD at the point b can be expressed as

$$\frac{1}{D} = \frac{1}{R_m + R_n} \tag{9}$$

The intercept of CD with the current i-axis is y_1, which is expressed as

$$y_1 = \frac{AR_m^2C_m}{(R_m + R_n)^2} \tag{10}$$

The intercept of the voltammogram with the current i-axis is y_2; therefore

$$\Delta y = y_1 - y_2 = 2y_1 \exp\left(-\frac{R_m V_0}{y_1 D R_n}\right) \tag{11}$$

Thus, without any time-wasted fitting procedures, all the membrane parameters can be calculated cycle by cycle from the on-line data acquire as follows:

$$\begin{aligned} R_m &= \frac{m}{m+1} D \\ C_m &= \frac{y_1}{A}\left(\frac{m+1}{m}\right)^2 \\ R_n &= \frac{1}{m+1} D \\ C_s &= \frac{h}{A} \\ m &= \ln\left(\frac{2y_1}{y_1 - y_2}\right) y_1 \frac{D}{V_0} \end{aligned} \tag{12}$$

where D, y_1, y_2, and $2h$ are acquired from on-line voltammetry.

In conclusion, the only parameters that need to be acquired directly for a recorded voltammogram of the s-BLM are D, y_1, y_2, and $2h$. From the relationship described in Eq. (12), it is now possible to calculate accurate values of the membrane electrical parameters for the s-BLM system. Here a novel method is proposed which can determine the properties accurately without any iteration (21). So the real-time measurement is realized to be suitable for the dynamic analysis.

Solid-Supported BLM Formation. Similar to the time-resolved spectrometry through which the transient molecular reaction can be observed optically, the dynamics of modified or unmodified BLMs, or reconstituted systems using the BLM, is studied electrically by the CV technique where the capacitance, resistance, membrane potential, and current peak are the fundamental parameters in determining the static or the dynamic change of the BLM system. Among these studies, the formation of the s-BLM is a valuable one in analyzing the BLM mechanics and its electrochemical reactions.

Here, the membrane capacitance and resistance are chosen as two principal parameters in monitoring the formation of unmodified s-BLMs. The time-resolved capacitance and resistance are measured following the model described above, based on the recorded s-BLM voltammogram. Table 3 gives the values of s-BLM electrical properties under different BLM-forming solutions. The unmodified s-BLMs were formed from various lipid solutions: 0.5% and 2% lecithin PC (PC = phosphatidylcholine) in n-decane. The triangle potential wave sweeps within ±100 mV, at sweeping rate of 50 mV/s. The setup, materials preparation and techniques for the formation of s-BLM have been described in the above section.

Dynamics of s-BLM Formation. The dynamics of the s-BLM should be studied to establish the membrane parameters of the s-BLM in stable stage (21). Figure 6 presents the time-resolved membrane resistance (a) and capacitance (b) during the s-BLM formation. Several successive stages are featured as shown (c), according to the mechanics of s-BLM formation described in a previous report (21). In Fig. 6, the continuous curves correspond to the forming solution of three different concentrations (curves 1, 2, and 3 for 0.5%, 2%, and 10% lecithin, respectively). The formation dynamics indicates that the higher membrane capacitance and resistance of static s-BLM (measured 600 s later) are obtained for the more concentrated solution. This phenomenon is in agreement with the fact that, as the concentration increases, the lipid molecules attached onto the unit area of solid surface (nascent surface of silver wire) become more dense (less solvent), and alter the dielectric constant and resistivity of the lipid bilayer.

Another important phenomenon is that the resistance as shown in Fig. 6(a) has the tendency shifting to the left with the increasing lipid concentration, while the capacitance [Fig. 6(b)] changes in the reverse direction (right shifting). With the denser packing, the lipid molecules quickly cover the freshly cut surface of the metallic wire, and less leak charge can go through the lipid-metal interface, as well as through the Plateau–Gibbs (P–G) border which is provided by the lipid solution between the Teflon coating and the metal wire. This causes the resistance rising in a shorter time for a more concentrated solution. But for the capacitance, though the denser lipid molecules block the current, their orderly parallel arrangement (perpendicular to the cut face of the metallic wire) becomes more difficult to reach the final state. This may be the reason for the capacitance "delay" in more concentrated lipid solutions.

The major principle of the CV technique is to impose an extra potential on the s-BLM in monitoring its dynamics. This influence is detected by the comparison of the "continuous" measurement and "point" measurement (also called nonimpact measurement). The point measurement is conducted through the single cyclic voltammogram recording at several predetermined time points while holding the s-BLM electrically isolated for a different period of time. In contrast, the continuous measurement is the cyclic recording without interruption during the entire formation. The result is reflected in Fig. 6(a, b) (cross-point curve 4) by the point monitoring for the 0.5% lecithin forming solution. The interval between two adjacent measurements is 60 s. The three continuous curves which have been described above are the result of continuous measurement. In Fig. 6(a) (compare curve 1 and the cross-point curve), little change is found regarding the effect of CV

Table 3. Electrical Properties of Solid Supported BLM (Diameter: 0.1 mm)

BLM Solution	R_m(MΩ · cm^2)	C_m(μF cm^{-2})	R_n(MΩ · cm^2)	C_s(pF)
Lecithin (0.5%) in n-decane	0.54	1.56	0.43	27
Lecithin (2%) in n-decane	1.74	0.63	0.40	31
Lecithin (10%) in n-decane	2.25	0.82	0.71	25

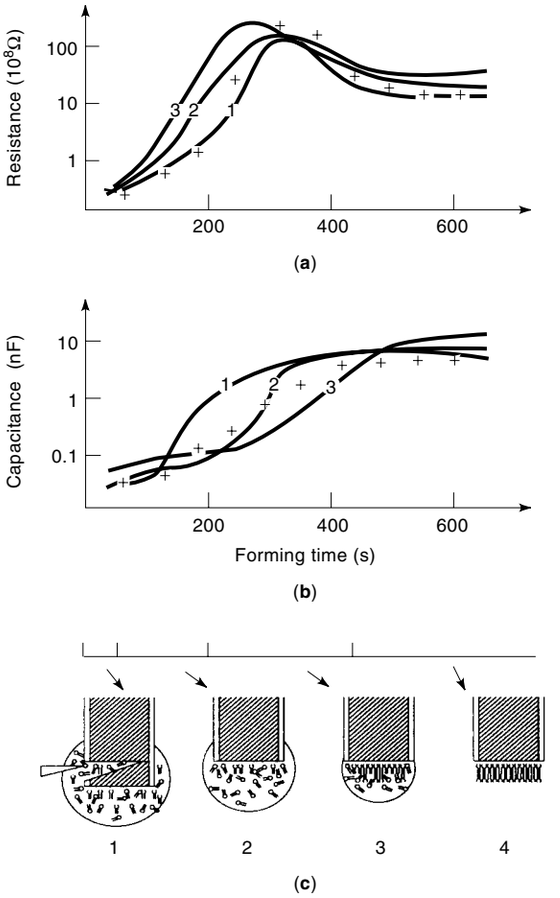

Figure 6. S-BLM formation dynamics: (a) Time-resolved membrane resistance during the formation process (curves 1, 2, and 3, continuous monitoring for 0.5%, 2%, and 10% lecithin solutions, respectively; "+" denotes point monitoring without CV potential effect): (b) Time-resolved membrane capacitance during the formation process (curves 1, 2, and 3, continuous monitoring for 0.5%, 2%, and 10% lecithin solutions, respectively; "+" denotes point monitoring without CV potential effect). (c) Characteristic stages of the s-BLM formation monitored by the membrane capacitance: (1) The tip of Teflon-coated platinum wire is cut off with a sharp blade while immersed in a lipid solution; (2) the newly cut surface of the wire attracts the polar groups of the lipid molecules, and thus a monolayer of lipid molecules is irreversibly adsorbed onto the tip of the wire, while the hydrophobic hydrocarbon chains are in contact with the lipid solution in air; (3) upon immersion of the wire into the aqueous solution, the lipid coating adhering to the metal surface will thin down spontaneously to a lipid bilayer, with the hydrocarbon chains of the two layers facing one another and the polar heads of the second layer of lipid molecules exposed to the water; (4) a self-assembled BLM adsorbed on a metal support has been formed.

on the resistance, but the capacitance right shift in Fig. 6(b) (comparing curve 1 and the cross-point curve) is detected for point monitoring. The polar groups of lipid molecules are supposed to be charged for this behavior. In the initial stage of BLM formation, the lecithin PC polar molecules are in a random state. In the case where no CV potential is imposed on the BLM, the preferred self-organization is being completed only through the attraction between the highly hydrophilic nascent metal surface and the polar groups of the lipid molecules. However, the detecting cyclic potential wave arouses an additional alternative electrical field. The "in field" molecules are forced by the sweeping voltage to accelerate their orientation, which as a result shortens the time for the bilayer formation on the nascent metal surface.

According to the capacitance monitoring of the s-BLM formation (see Fig. 6), in general, there are about four characteristic stages that can be distinguished from the initial "cutting operation" to the final formation of the s-BLM. These stages are as follows:

1. At the beginning, the capacitance fluctuates at random for a few seconds because the tip of the Teflon-coated metallic wire has just been cut off with a sharp blade while immersed in a lipid solution.
2. During the next few seconds, the capacitance is relatively low due to the droplet of the BLM forming solution on the newly cut surface of the metallic wire. Rapidly a monolayer of lipid molecules is irreversibly adsorbed onto it.
3. The capacitance tends to increase after upon immersion of the wire into the aqueous solution. The lipid droplet on the tip becomes thinner and leads spontaneously to form a lipid bilayer. Moreover, the potential of CV also tends to speed up the s-BLM formation.
4. As the s-BLM adsorbed on a metal support has been formed, the capacitance becomes stable, though sometimes it fluctuates slightly due to the transfer of the solvent and of the excess lipids to the aqueous phase as well as to the P–G border [see Fig. 1(f)]. In fact, all the capacitance values in Table 3 have been measured in this stage.

Self-Assembled Lipid-Bilayer-Based Biosensors. It should be pointed out that, first of all, unmodified lipid bilayers (i.e., BLMs formed from common phospholipids or oxidized cholesterol dissolved in *n*-octane) in 0.1 M KCl will typically have the following electrical properties: R_m greater than $10^8\ \Omega \cdot cm^2$, C_m of about 0.4 $\mu F/cm^2$, E_m about 0, V_b about 200 $\pm$ 50 mV, and current–voltage (I–V) curves obeying Ohm's law. However, the electrical properties of BLMs can be drastically altered by incorporating a host of materials such as pigments, dyes, polypeptides, membrane proteins, organic metals, and semiconductor particles (3,4).

Advances of Self-Assembled Lipid-Bilayer-Based Biosensors

There have been a number of reports on self-assembled molecules or structures described as advanced materials or smart materials. Without question, the inspiration for this exciting work comes from the biological world, where the lipid bilayer of cell membranes plays a pivotal role. Insofar as planar lipid bilayers are concerned, these are evidenced by self-assembled lipid bilayers, photoelectric effects in pigmented BLMs, and TCNQ-based BLM rectifiers, and they most recently supported BLMs on interdigitated structures as biosensors by microelectronics techniques (22,23). Our approach to materials science research is a biomimetic one and is centered on experimental BLMs in that the membranes can function in such important processes as electron transfer, signal transduction, and cellular environmental sensing (3,4). Specifically, the fol-

lowing experiments, some of which are in progress, are delineated below.

Incorporation of Ferrocene in s-BLMs. To test the versatility of s-BLMs as a "smart material," an amperometric sensor was constructed for ferri-/ferrocyanide ions. The results have shown that (1) ferrocene can be very easily immobilized in the lipid bilayer on the tip of a metallic wire (s-BLM) system and (2) ferrocene in an s-BLM on a Pt support increases the sensitivity by about two orders of magnitude for potassium ferri-/ferrocyanide ions compared with that of a bare platinum electrode. This demonstrates that the s-BLM system offers a novel approach to the electrode modification by incorporation of compounds within a lipid bilayer (24–26).

Hydrogen Peroxide-Sensitive s-BLMs. The insertion of appropriate active molecules (modifiers) into the matrix of the lipid bilayer should be able to impart functional characteristics to s-BLMs. We chose TCNQ and dipyridyl-tetrathiafulvalene (DP-TTF) because of their properties as typical electron acceptor and donor molecules, respectively. It was found that DP-TTF not only improved the stability but also increased the range of s-BLM's sensitivity to hydrogen peroxide. In contrast, TCNQ-containing s-BLMs did not show much response to H_2O_2 (25).

Modified-BLMs as pH Sensors. The hydrated hydrogen ion (H_3O^+) is crucial to the functioning of cellular processes because it plays a leading role in enzyme catalysis and membrane transport. To test our concept, we incorporated a number of quinonoid compounds (chloranils) into s-BLMs and found that, indeed, s-BLMs containing either tetrachloro-*o*-benzoquinone (TC*o*BQ) or tetrachloro-*p*-benzoquinone (TC*p*BQ) responded to pH changes whose potential measurements exhibit a nearly theoretical slop (27,28). In this connection, mention should be made that s-BLMs formed from a glycerol dioleate (GDO) lipid solution containing polypyrrole have been found to be sensitive to pH with a Nerstian slope of 57 mV/decade hydrogen ion concentration as compared with unmodified s-BLMs (slope $\approx$ 20 mV). These new pH-sensitive s-BLMs offer prospects for a ligand-selective probe development using microelectronics technologies.

Modified s-BLMs as Ion Sensors. S-BLMs containing six different kinds of crown ethers were investigated using cyclic voltammetry. In particular, s-BLMs formed from a liquid crystalline aza-18-crown-6 ether and cholesterol-saturated *n*-heptane solution were found to be sensitive to K^+ in the concentration range 10^{-4} to 10^{-1} M with the theoretical Nernstian slope (29,30). The specificity for three alkali metal cations and NH_4^+ of these doped BLMs were also investigated. The order of specificity for most of these bis-crown ethers was found to follow hydrated radii of the cations, that is, $NH_4^+ > K^+ > Na^+ > Li^+$ (29). The results obtained with these s-BLMs compare favorably with conventional BLMs containing similar compounds, such as valinomycin and gramicidin (9). Concerning the gramicidin, it is well known that it forms ion channels. Cornell et al. (31) reported recently that a lipid-bilayer-based biosensor contains "sliding ion gates" made of gramicidin, and they claimed that a device can be designed for almost any analyte for which a receptor can be synthesized. The idea is again based on ligand–receptor interactions (3,4,26,32).

Recently we have investigated electron transfer across a BLM containing the so-called buckminsterfullerene C_{60}, which can act both as a mediator and a photosensitizer (see a later section on photoactive s-BLMs). Using the s-BLM, it is possible to construct a sensor probe sensitive to iodide ion and to investigate redox reactions across the lipid bilayer (33). The presence of C_{60} greatly strengthened the stability of the s-BLM and dramatically alters its electrical properties. Using the cyclic voltammetry technique, the results show that C_{60} embedded in the BLM acts as an excellent electron carrier/mediator and should be useful for electrochemical biosensor and molecular electronics device development. The cyclic voltammograms contain distinct redox peaks, which are not symmetrical. Further, the C_{60} in the lipid bilayer had the opposite effects on membrane resistance (R_m) and capacitance (C_m), respectively; it caused the R_m to decrease and C_m to increase. The presence of iodine in conjunction with C_{60} in the s-BLMs further accentuated the effects. It is apparent that both C_{60} and iodine, when embedded in BLMs, facilitate electrical conduction, thereby lowering the R_m. Since the dielectric constant, ϵ, of a typical, unmodified BLM is about 2, the presence of C_{60} and iodine should exert a great influence on ϵ. Since C_m depends on a number of factors such as the surface charge of the BLM, the nature of hydrocarbon chains, and embedded modifiers, an increase in ϵ, and consequently in C_m, is therefore expected. The modified probe increased the detection limit for iodide ion by 100 times, with a linear response in the range from 10^{-8} M to 10^{-2} M. Thus, C_{60} facilitates the discharge of I_3^- at the metal surface, which demonstrates clearly that the embedded C_{60} is indeed an excellent electron mediator (33).

Modified s-BLM as Molecular Sensors. Many authors have reported sensors for the detection of glucose using glucose oxidase (34). Interestingly, using s-BLMs containing redox compounds and electron mediators but without the enzyme, glucose was detected in the buffered solution. The results are preliminary and further experiments are in progress. If a highly conjugated compound such as TCNQ is incorporated in the s-BLM forming solution, the resulting s-BLM was able to detect the presence of ascorbic acid, which is consistent with the findings obtained with conventional BLMs (35).

Molecular Recognition in an s-BLM. S-BLMs can be employed for embedding a number of compounds such as enzymes, antibodies, protein complexes (channels, receptors, membrane fragments or whole cells), ionophores, and redox species for the detection of their counterparts, respectively, such as substrates, antigens, hormones (or other ligands), ions, and electron donors or acceptors (9). The antigen–antibody reaction can be undertaken by using s-BLMs as the probe with electrical detection. The antigen, hepatitis B surface antigen (HBs-Ag) was incorporated into an s-BLM, which then interacted with its corresponding antibody (HBs-Ab or monoclonal antibody) in the bathing solution. This Ag–Ab interaction resulted in some dramatic changes in the electrical parameters (conductance, potential, and capacitance) of s-BLMs. The magnitude of these changes was directly related to the concentration of the antibody in the bathing solution

Table 4. Effect of Concentrations of HBs-Ab on the Resistance of s-BLMs

[HBs-Ab] (ng m^{-1})	$R_1(\times 10^8)$ ($\Omega \cdot cm^2$)	$R_2(\times 10^8)$ ($\Omega \cdot cm^2$)	$\log R_1/R_2$
100	6.05	0.429	3.15
80	6.14	0.504	3.09
60	6.04	1.63	2.59
50	5.07	2.43	2.35
40	5.15	5.50	2.01
30	5.55	8.80	1.80
20	6.30	28.1	1.35
10	6.30	51.2	1.09
1	6.50	162	0.604

(see Table 4). The linear response was very good from 1 to 50 ng ml^{-1} of antibody, demonstrating the potential use of such an Ag–Ab interaction via the s-BLM as a transducing device (36).

Electron Transfer Experiments in s-BLMs. Research in the field of electron transfer processes in BLMs was first conducted in the late 1960s to understand the primary step in natural photosynthesis (2,37). It was discovered that a light-driven electron transfer process between donor and acceptor species can occur across the thickness of a pigmented BLM. This finding has subsequently led to the view that the reaction center of natural photosynthesis functions similarly to that of a photovoltaic device of molecular dimensions (12). In the mid-1980s, electron transfer in the dark was seen in BLMs doped with either organic "metals" or semiconducting nanoparticles formed in situ (18). These phenomena were explained in terms of light-induced charge separation, field-driven charge transport, and subsequent redox reactions on opposite sides of the BLM. In the absence of light, the theory of electron tunneling was invoked (see above on TCNQ or TTF containing BLMs). When an s-BLM doped with Zn-phthalocyanine was excited by light, a voltage and a current were recorded, with the action spectra closely parallel to the absorption spectrum of the photon absorber (16). Thus we have shown that a pigmented s-BLM can function as a light transducer or photon-activated switch or detector (33,38).

S-BLMs Deposited on Piezoelectric Quartz Crystals. Smell and taste (olfaction and gustation) are among living organisms' two most vital sensing systems, the biophysics of which have been increasingly elucidated at the molecular level (7,22). Here again the crucial receptors are BLMs. In the preliminary experiments, several kinds of BLMs were successfully deposited on AT-cut quartz resonators (7,39). These were verified by observing frequency (f_m), potential (E_m), capacitance (C_m) and I–V curves. Frequency change (versus that in air) ranged from 9 kHz to 16 kHz, and in cyclic voltammetry experiments no redox peaks could be observed or the peaks were largely damped in the presence of $Fe(CN)_6^{3-}$. E_m and C_m also showed characteristic values. But the exact values of these parameters were found to be related to the lipid solution, the pH of the bathing solution, and the scan time of voltammograms. If the BLM failed to form or was broken, obvious changes in these parameters were observed. In this case, f_m increased several kilohertz (to about 6 kHz, which corresponds to that induced only by pure viscous loading); C_m and E_m also increased and characteristic redox peaks were observed. Our findings show that BLMs can be formed on piezoelectric quartz crystals and that the piezoelectric techniques can be applied as a powerful tool to characterize the s-BLM system.

S-BLMs on Interdigitated Structures by Microelectronics Techniques. The fact that a lipid bilayer structure can be self-assembled on a solid substrate is intriguing. This novel manner of lipid bilayer formation overcomes two basic obstacles in the way of practical utilization of the BLM structure, namely: (a) its stability and (b) its compatibility with a standard microelectronics technology. As has been repeatedly demonstrated by us and others (23,40), the solid s-BLM system not only possesses the advantages of a conventional BLM structure, but gains new important properties, besides its long-term stability, such as (1) an anisotropic, highly ordered, yet very dynamic liquid-like structure and (2) two asymmetric interfaces, one of which is metallic. With this metallic connection, this type of probe solves the interfacing problem and is applicable to microelectronics technology. On this last-mentioned property, we have extended the experiment described above to the interdigitated structures (IDS). IDS are finger-like electrodes made by microelectronics technologies and used in microchip applications (23). By forming s-BLMs on IDS made of platinum with a window of 0.5 mm $\times$ 0.5 mm, we obtained the following interesting results. First, when an IDS coated with a BLM was formed from asolectin, it responded to pH changes with only a (15 $\pm$ 2) mV/decade slope. The conductance of s-BLMs on the IDS was about 50 times higher than the usual s-BLMs. Second, when an IDS coated with a BLM formed from asolectin was doped with either TC*o*OBQ or TC*p*BQ, the pH response was linear with a slope close to the theoretical value (25,27). This type of structure (i.e., s-BLM on interdigitated electrodes) can be used to investigate ligand–receptor contact interactions.

S-BLMs on an IDS can be manufactured using microelectronics technologies which already exist without the explicit need for special modification. This finding is viewed as a major "breakthrough" in the biosensor development. In this connection it should be mentioned that experiments on IDS chips modified with a BLM are based on a common basic aspiration—that is, to self-assemble a lipid bilayer containing membrane receptors, natural or synthetic, so that a host of physiological activities, such as ion–molecular recognition, can be investigated (7,23). At the molecular level, most of these activities may be termed collectively as the receptor–ligand contact interactions. The structures which are being reconstituted are inherently dynamic. Receptors and ligands in such close contact will normally vary as a function of time, frequently resulting in non-linear behavior. With an IDS chip modified with a BLM, we now have at last a most unique system for extensive experimentation which will be limited only by our imagination. Thus, insight gained from these studies will guide the preparation of functional BLMs on IDS support. Our aim is to take advantage of microelectronics techniques and apply them to the biochemical and neuroscience research.

Photoelectric Effects in Planar s-BLMs and sb-BLMs. Fullerenes (e.g., C_{60}) have been of great interest in materials science in the last decade. Our interest in fullerenes as a BLM mod-

ifier is owing to their most unique properties. Unmodified C_{60}, for example, is a water-insoluble and highly conjugated hydrophobic compound; it behaves as a good electron mediator and as an *n*-type semiconductor (bandgap = 1.6 eV). Hence, the lipid bilayer is an ideal environment for the compound to reside. The C_{60}-containing s-BLM, considered basically as a "molecular device," is a light-sensitive diode capable of photoinduced charge separation which undergoes redox reactions across the substrate–hydrophobic lipid bilayer–aqueous solution junctions.

Concerning photoactive compounds in BLMs, mention should be made about electron transfer processes in the membranes of photosynthesis and mitochondrial respiration, both of which have been reported in a number of BLM studies (2,8,12,15,37,41). In the 1980s, electron transfer was demonstrated in BLMs. The phenomena were explained in terms of the light-induced charge generation and separation, field-driven charge transport, and subsequent redox reactions on opposite sides of the BLM. In the absence of light, the theory of electron tunneling was invoked (12,20). When an s-BLM doped with Zn-phthalocyanine was excited by light, a voltage and a current were recorded, with the action spectrum paralleled closely to that of the absorption spectrum of the pigment (9,16). This provides direct evidence that the light-induced currents are indeed due to the photoactive compound embedded in the BLM. Thus, a pigmented s-BLM can function as a light transducer or photon-activated switch or detector. In this connection, the experiments reported by Rolandi et al. (42), Yonezawa et al. (43), Lamrabte et al. (44), Yamaguchi and Nakanishi (45), Bi et al. (46), Ikematsu et al. (47), and more recently by Gruszecki et al. (48) should be of great interest.

Molecular Electronics and Lipid-Bilayer-Based Biosensors

Molecular electronics makes uses of materials at the molecular level in which the species retain their separate identities. As a result, the properties of such materials depend on the molecular arrangement, properties, and interactions. Theory seeks to guide the design and synthesis of effective molecular materials (13). It does so by analysis, interpretation, and prediction, leading to the development and evaluation of concepts, models, and techniques (22). The role of theory in treating molecular properties (mainly by molecular orbital methods) and arrangement (by electromagnetic or quantum-mechanical approaches) is of importance. When these factors are combined, the material properties can be treated more successfully in cases where the interactions are not essential for the existence—for example, in nonlinear optics as opposed to electronic transport properties (49).

The major advantage of molecular electronics with a lower limit on the order of micrometers is the further development of lithographic techniques. The changed physical properties in the submicroscropic region are the major obstacles to further miniaturization in the semiconductor technologies. The physical border for the silicon technologies is about 100 nm, because one cannot overcome the characteristic lengths such as diffusion, Debye, and tunnel lengths. With still smaller dimensions, we enter the realm of biological and molecular systems. Although biotransducers function much slower than silicon-based devices and are not very reliable, they are extremely efficient. Also, despite their disadvantages, nature's molecular devices function more generally and are superior to technical computers or sensors. In contrast to macromolecular biological systems, the main advantage of molecular devices, purportedly, is their relatively simple construction. In this sense, molecular devices may be readily constructed, and they are always easily accessible experimentally from a quantitative point of view (17).

The main elements of molecular electronics are molecular wire, conducting material, molecular-specific transducers of signals similar to the particles, and molecular switches, memories, emitters, detectors, and so on. The flux of information between the molecules can be released in many ways. One of the most important is the transfer of individual charges in terms of electrons, holes, or hydrogen ions, or of other shapes similar to the elements, like solitons, soliton waves, or excitons. Molecular switches may be optical, electrical, magnetic, or thermally reversible systems, such as photochrom-salicylidenanilin. Storage of information in a molecular system can be realized through a change in the electronic as well as geometric structures of the molecules in reversible thermal reactions—for example, conformational or configurational changes upon replacement of hydrogen or protons (22,49).

FUTURE PROSPECTS

In recent years, the development of biosensor configurations has been concentrated largely around the design of the transducer used. Further researcher's attention, however, should be focused on the mechanism of molecular recognition and catalysis. The fundamental properties of the device must be better understood in order to optimize critical factors such as response time, selectivity, and stability. Immobilization technologies and new membrane materials may basically change the present performance of biosensors.

The key advantage of molecular and biomolecular computing is specificity (49). The large number of variations that are possible with organic polymers such as poly (*N*-isopropylacrylamide as a "linking arm" in the protein ligand recognitions, and artificial photochrome and photorefractive material for future photon information storage and optical computer) allows for fine tuning of electronic motions to a much greater extent than is possible with organic materials. In biological molecules, certain configurational motions are comparable in significance to electronic motions. This is certainly the case in all conformation-based recognition processes. For example, the energy-loan model is applicable to many conformational switching processes based on the "lock-key"-type interactions of macromolecules. This could include self-assembly processes, protein folding, and various motions of biological macromolecules, which are generally attributed to fluctuation phenomena. The electronic instability in the energy-loan model may be thought of as a mechanism for amplifying the effects of fluctuations.

The switching process based on the energy-loan mechanism can mediate important forms of signal processing within biological cells. The enzymatic recognition is itself a basic form of information processing. When proteins and other macromolecules are combined into highly integrated complexes, they become possible for conformational switching processes to propagate over significant distances. The cytoskeleton is a good candidate for such a signal-processing network (49). One

hypothesis is that conformational changes propagate through the cytoskeletal network by the energy-loan mechanism, with the conformation change of component macromolecules inducing the required lattice distortion in neighboring macromolecules. Such propagating conformation changes would play a role in transport processes in the cell, and it is likely that they would play a role in information processing as well.

One of the most delicate "molecular wires" is the so-called conjugated hydrocarbon chain, which is the best represented through the chain of carbon atoms in polymers. Most organic polymers are well-known insulators. However, polyacetylene $(CH)_x$, polydiacetylene, and polysulfinitride $(SN)_x$, with their conjugated double bonds, are semiconductors or superconductors. Such conjugated systems form the group of organic conductors and semiconductors. The most important organic electroactive polymer is polyacetylene. The foundation of the electron–hole pairs and the positive and negative charges are quite well known. In the outer electric field, the electron and the hole are accelerated in opposite directions. This properties can be used in optical switches for switching on and off the flux of information. Combining molecular "wire" and switchable molecules could lead to the construction of electronic systems based on molecules. Present research is oriented toward discovering peptides/proteins that can transduce electrical current or exist in two-electrical states. These could lead to future "biochips." Research on biochips could lead not only to a better understanding of higher nerve function, but also to the foundation of qualitative computer systems that could provide many of the activities currently performed only by the human brain.

Biochips can be considered, therefore, as highly sophisticated biosensors. The unique properties of biochips are their analog and digital computing potentials, self-perpetuating and potentially self-repairing. Biochips hold promise in a variety of applications such as bionic implants, memory-intensive systems, image processing and storage, artificial intelligence, language processing, and molecular computers. For instance, the analog capability of biochips could enable the creation of "artificial intelligence." As such, biochips are at a very early stage of research and development (22,49). As with biosensors, the current problem is our inability to produce uniform high-activity, stable biomolecular layers and their associated transduction systems. These problems notwithstanding, it seems likely that the initial application of biochips as advanced biosensors, based on the ligand–receptor contact interaction, may be in the clinical setting, where they could serve as automated control devices for drug delivery (17). It also appears probable that in order to extend the capabilities of present silicon-only systems, hybrid biochips and silicon-chip devices would be first produced for computing and memory-intensive systems. The key to the successful application of biochips will be to fill places that are not well-served by current silicon chip technology. Thus, the future development of biochips requires the successful technologies of stable biomolecule embedding and immobilization, biotransduction, and molecular lithography. Several urgent problems to be solved are biologically based amplification, molecular switching, electron transport, and memory function. In the coming decade, the answers to some of these problems will undoubtedly be found. In this connection, the development of lipid-bilayer-based sensors and biological electronic devices seems to be a logical first step. With the BLM systems, especially s-BLMs, we now have an experimental approach for testing some of the new ideas in the development of sensors for practical applications (1,23,31,52).

BIBLIOGRAPHY

1. R. F. Taylor and J. S. Schultz (eds.), *Handbook of Chemical and Biological Sensors,* Philadelphia: IOP Publishing, 1996.
2. H. T. Tien, *Bilayer Lipid Membranes BLM: Theory and Practice,* New York: Marcel Dekker, 1974.
3. H. T. Tien, Self-assembled lipid bilayers for biosensors and molecular electronic devices, *Adv. Mater.,* **2**: 316–318, 1990.
4. H. T. Tien, S. H. Wurster, and A. L. Ottova, Electrochemistry of supported bilayer lipid membranes: Background and techniques for biosensor development, *Bioelectrochem. Bioenerg.,* **42**: 77–94, 1997.
5. F. T. Hong (ed.), *Molecular Electronics: Biosensors and Biocomputers,* New York: Plenum, 1989.
6. K. L. Mittal and D. O. Shah (eds.), *Surfactants in Solution,* New York: Plenum, 1989, vol. 8, pp. 133–178; 1992, vol. 11, pp. 61–80.
7. A. L. Ottova et al., Self-assembled and supported BLMs as an adaptive material for biotechnology, *SPIE's 1996 Symp. Smart Structures Mater.,* 1997, vol. 2716, pp. 144–151.
8. G. Dryhurst and K. Niki (eds.), *Redox Chemistry and Interfacial Behavior of Biological Molecules,* New York: Plenum, 1988, pp. 529–556.
9. A. L. Ottova and H. T. Tien, Bilayer lipid membranes: An experimental system for biomolecular electronic devices development, *Prog. Surf. Sci.,* **41** (4): 337, 1992.
10. M. Rosoff (ed.), *Vesicles,* New York: Marcel Dekker, 1996, pp. 49–77.
11. C. V. Boys, *Soap Bubbles: Their Colours and the Forces Which Mould Them,* New York: Dover, 1959.
12. S. G. Davison (ed.), *Prog. Surf. Sci.,* **23**: 317, 1986; **30**: 1, 1989.
13. I. B. Ivanov (ed.), *Thin Liquid Films: Fundamentals and Applications,* New York: Dekker, 1988.
14. J. R. Harris and A.-H. Etemadi (eds.), *Artificial and Reconstituted Membrane Systems, Subcellular Biochemistry,* Vol. 14, New York: Plenum, 1989, p. 97.
15. M. Blank (ed.), *Biomembrane Electrochemistry, Advances in Chemistry Series,* No. 235, Washington, DC: ACS, 1994.
16. A. L. Ottova et al., Self-assembling bilayer lipid membranes on solid support: Building blocks of future biosensors and molecular devices, in R. R. Birge (ed.), *Molecular Electronics and Bioelectronics, Advances in Chemistry Series,* No. 240, Washington, DC: ACS, 1994.
17. J. R. Bourne (ed.), Lipid bilayer-baed sensors and biomolecular electronics, *Biomed. Eng.,* **18** (5): 323, 1991.
18. J. Kutnik and H. T. Tien, Application of voltammetric techniques to membrane studies, *J. Biochem. Biophys. Met.,* **11**: 317–326, 1985; J. Kutnik and H. T. Tien, Apparatus and techniques for measurement of electrical properties of bilayer lipid membranes BLM, *J. Electrophysiol. Tech.,* **14**: 211–221, 1987.
19. S. Kalinowski and Z. Figaszewski, A four-electrode potentiostat-galvanostat for studies of bilayer lipid membranes, *Meas. Sci. Technol.,* **6**: 1043–1055, 1995.
20. H. T. Tien, Cyclic voltammetry of electron-conducting bilayer lipid membranes, *Bioelectrochem. Bioenerg.,* **13**: 299–316, 1984; **15**: 19–38, 1986.
21. L. Q. Gu et al., A new method for the determination of electrical properties of supported bilayer lipid membranes by cyclic voltammetry, *Bioelectrochem. Bioenerg.,* **39**: 275–283, 1996.

22. H. T. Tien et al., Bilayer lipid membrane (BLM): An experimental system for biomolecular electronic device development, *J. Mol. Electron.,* **4**: s1, 1988.
23. V. Tvarozek et al., Thin-film microsystem applicable in (bio)-chemical sensors, *Sens. Actuators: B. Chemical,* **19**: 597, 1994.
24. H. T. Tien et al., *Anal. Lett.,* **26**: 819, 1993.
25. W. Liu et al., in R. L. Opila et al. (eds.), *Polymer / Inorganic Interfaces,* 304, Pittsburgh, PA: Materials Research Society, 1993.
26. A. Leitmannova-Ottova and H. T. Tien, Receptor–Ligand Contact Interactions Using Self-Assembled Bilayer Lipid Membranes, in E. P. George et al. (eds.), *Materials for Smart Systems, The MRS Symp. Proc. Series, reviewed,* vol. 360, Pittsburgh, PA: Material Research Society, 1995.
27. W. Ziegler et al., in V. Tvarozek and S. Nemeth (eds.), *Proc. 7th C-S Conf. Thin Films,* pp. 304–311.
28. A. L. Ottova, D.-L. Guo, and H. T. Tien, *Proc. Conf. Smart Structures Mater.,* Orlando, FL, 1995.
29. (a) Z. H. Tai, L. Cun, and C. Z. Yang, Pt supported BLMs modified by ferrocene and its derivatives, *Mol. Eng.,* **2**: 215–220, 1994. (b) Z.-H. Tai et al., Molecular assemblies of diszafluorenone Schiff-base amphiphiles: Bilayer membrane and its electrochemical oscillations, *Mol. Eng.,* **3**: 285–291, 1994.
30. Y.-E. He et al., Crown ether-modified bilayer lipid membranes on solid support as ion sensors, *Analytical Lett.,* **28** (3): 443, 1995.
31. B. A. Cornell et al., A biosensor that uses ion-channel switches, *Nature,* **387**: 580–583, 1997; B. Raguse et al. Tethered bilayer lipid membranes: Formation and ionic reservoir characterization, *LANGMUIR* **14**: 648–659, 1998.
32. A. L. Ottova et al., Supported BLMs: Biomembrane models and biosensor applications, *Supramol. Sci.,* **4**: 101, 1997.
33. H. T. Tien et al., Electronic processes in supported bilayer lipid membranes (s-BLMs) containing a geodesic form of carbon (fullerene C_{60}), *Bioelectrochem. Bioenerg.,* **42**: 161, 1997.
34. J. Kotowski, T. Janas, and H. T. Tien, Immobilization of glucose oxidase on a polypyrrole–lecithin bilayer lipid membrane, *J. Electroanal. Chem.,* **253**: 277, 1988.
35. X.-D. Lu, A. L. Ottova, and H. T. Tien, Biophysical aspects of agar-gel supported bilayer lipid membranes BLMs: A new method for forming and studying planar BLMs, *Bioelectrochem. Bioenerg.,* **39**: 285–289, 1996.
36. L. G. Wang, Y.-H. Li, and H. T. Tien, Electrochemical transduction of an immunological reaction via s-BLMs, *Bioelectrochem. Bioenerg.,* **36**: 145, 1995.
37. P. Bianco and J. Haladjian, Control of the electron transfer reactions between c-type cytochromes and lipid-modified electrodes, *Electrochem. Acta,* **39**: 911–916, 1994.
38. H.-M. Wang et al., *Bull. Electrochem.,* **12**: 496, 1996.
39. A. L. Ottova et al., in M. Alper, et al., (eds.), Biomolecular materials by design, *Proc. Mater. Res. Soc.,* Fall Meeting, 1993, p. 330.
40. A.-N. Sun et al., Research on electrical properties of amphiphilic lipid membranes by means of interdigital electrodes, *Mater. Sci. Eng. C,* **2**: 159–163, 1995.
41. L. Song, Y.-J. Xiao, and X.-X. Gao, A simple method to form an asymmetric BLM and its enhancement of photoelectric responses, *Langmuir,* **12**: 4303–4304, 1996.
42. R. Rolandi, D. Ricci, and O. Brandt, *J. Phys. Chem.,* **96**: 6783, 1992.
43. Y. Yonezawa, H. Fujiwara, and T. Sato, *Thin Solid Films,* **210/211**: 736, 1992.
44. A. Lamrabte et al., *Bioelectrochem. Bioenerg.,* **27**: 449, 1992.
45. H. Yamaguchi and H. Nakanishi, Photoresponsive planar bilayer lipid membranes containing azobenzene amphibilic derivatives, *Sens. Actuators B,* **13/14**: 376, 1993.
46. Z.-C. Bi et al., *J. Photochem. Photobiol. A: Chem.,* **77**: 37, 1994.
47. M. Ikematsu et al., *J. Electrochem.,* **403**: 61, 1996.
48. W. I. Gruszecki, A. Wardak, and W. Maksymiec, *Photochem. Photobiol. B Biol.,* **39**: 265, 1997.
49. F. L. Carter, R. E. Siatkowski, and H. Wohltjen (eds.), *Molecular Electronic Devices,* Amsterdam: North-Holland, 1988.
50. H.-P. Yuan, A. Ottova-Leitmannova, and H. T. Tien, An agarose stabilized BLM: A new method for forming bilayer lipid membranes, *Mater. Sci. Eng.,* **C4**: 35–38, 1996.
51. H. T. Tien, BLM-based electrochemical biosensors, *J. Clin. Lab. Anal.,* **2**: 256–264, 1988.
52. H. T. Tien and A. L. Ottova, Supported planar lipid bilayers, s-BLMs, as electrochemical biosensors, *Electrochim. Acta, J. Int. Soc. Electrochem.,* 1998, in press. H. T. Tien and A. L. Ottova, From self-assembled bilayer lipid membranes BLMs to supported BLMs on metal and gel substrates to practical applications, *Colloids Surfaces A: Physicochem. Eng. Aspects,* **143**, 1998.

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INTELLIGENT COMPUTER AIDED INSTRUCTION.

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