

Accounts

Design and Application of Ion-Channel Sensors Based on Biological and Artificial Receptors

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This paper reviews approaches to chemical sensing with receptor-modified electrodes that mimic the function of natural membranes containing biological and synthetic receptor channels. We highlight different types of ion-channel sensors, focusing on the working principle and the fundamental aspects, such as sensitivity and selectivity. It is demonstrated that biological receptors such as glutamate receptor ion channels and gramicidin, embedded in bilayer lipid membranes, have the potential as sensing elements for ion channel sensors with excellent sensitivity and selectivity. In addition, higher selectivities by improved receptor desigh, taking into account the particular advantages of interfacial analyte recognition at LB membranes and SAMs, are also shown with ion-channel sensors for antibodies, protamine, heparin, abasic DNA sites, and oligonucleotides.

All cell membranes share a common structural organization, i.e., bilayers of phospholipid with associated membrane proteins, which are responsible for many specialized functions. Some proteins act as receptors that respond to external signals, activating a series of intracellular signaling cascades, and some participate in transporting ions and molecules across the cell membrane.[1] All transmembrane and intracellular signaling processes are initiated by the binding of signaling ions and molecules to their receptors in bilayer membranes. Mimicking the principle of such natural sensor systems has been the objective of many recent studies in sensor development.[2,3]

A variety of new electrochemical sensors have been reported, including ion-channel[4–11] or ion-channel mimetic sensors,[12–15] active transport membrane electrodes,[16–19] ion-channel switch biosensors,[20–22] and micro bilayer lipid membrane sensors.[4,15] Their responses are based on transmembrane signaling, such as changes in membrane permeability[6–10,12–15,17,23] and membrane potentials.[16,24,25] In addition, advances in the molecular design of receptors and membranes for bio-sensors and biomimetic sensors extended the range of targeted analytes to electroinactive ones; the analytes do not necessarily need to be electroactive. Instead, electrochemically inactive analytes can be detected with these sensors by virtue of their working principles.

A biomimetic sensor whose working principle is similar to transmembrane signaling displayed by ligand-gated ion-channels in biological membranes was first reported in 1987.[12] The surface of a glassy carbon electrode was coated by means of the Langmuir-Blodgett technique with a thin layer of a synthetic lipid with a phosphate headgroup, and permeation of electroactive marker ions added to the sample solution was regulated by the binding of a stimulus (Ca^{2+}) to the negatively charged lipid. The sensor was called the ion-channel sensor because its working principle was similar to the control of transmembrane ion transport by ligand-gated ion-channels (Fig. 1). A considerable number of ion-channel sensors have been developed based on various artificial receptors for inorganic ions, organic ions and bioactive substances, as reviewed in recent articles.[14]

Although there has been more interest in artificial receptors, biological ion-channels like glutamate receptor ion-channels in combination with bilayer lipid membranes[4–10] and biological material such as DNAs,[26,27] DNA/enzyme,[28] oligonucleotides,[29] and oligopeptides[30] directly attached to the electrode surface have also attracted attention for designing ion-channel sensors. Recently, new modes of ion-channel sensors by using the channel former gramicidin linked to a receptor in a supported lipid bilayer membrane or by using gramicidin itself as

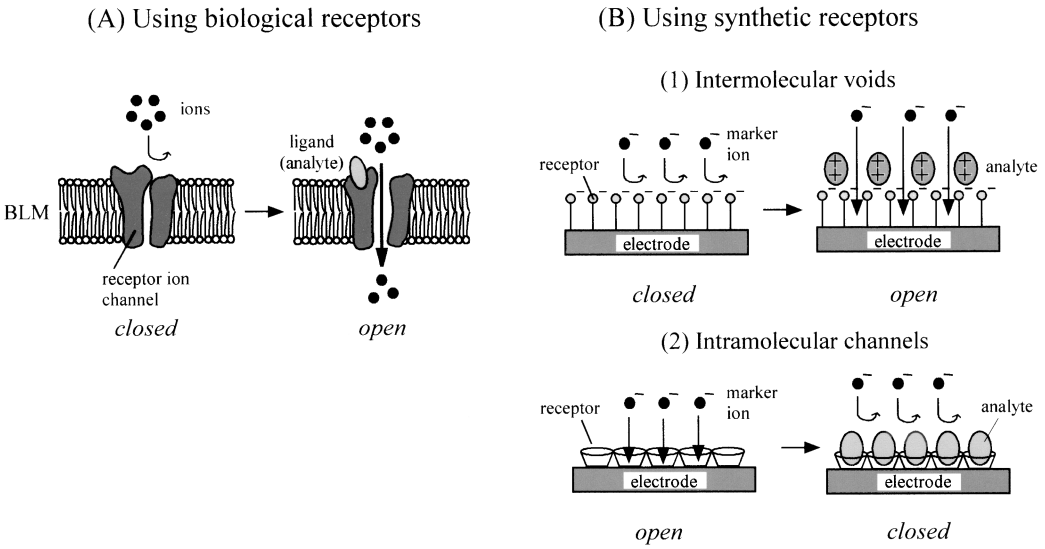

Fig. 1. Principles of ion-channel sensors based on (A) biological receptors and (B) synthetic receptors.

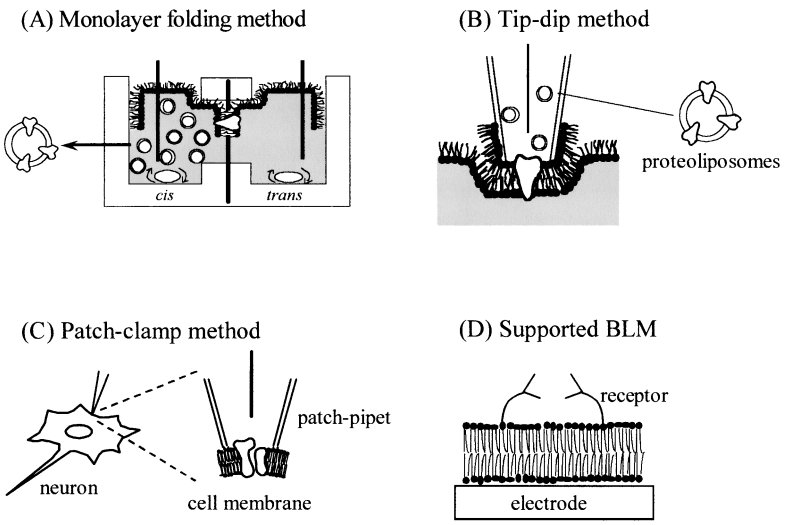

Fig. 2. Approaches for constructing BLM-based sensors.

a molecular signal transducer incorporated in a bilayer membrane have been reported.[20–22]

In this paper, we highlight different types of ion-channel sensors based on biological and artificial receptors, mostly reported by us, and focus on the working principle and the fundamental aspects, such as sensitivity and selectivity, of these sensors.

Ion-Channel Sensors Based on Biological Channels

Biological ion-channels are proteins that form open pores in response to specific chemical stimuli (ligand-gated channels and G-protein-mediated channels), changes in electric potential (voltage-gated channels) or mechanical stress (stretch-sensitive channels), allowing ions of the appropriate size and charges to pass.[1] For the design of ion-channel sensors based on biological receptors, ligand-gated ion-channels have attracted more interests, because the opening event is regulated by specific chemical stimuli (Fig. 1A). The approach based on ligand-gated channels utilizes isolated proteins from biological cells. The proteins are incorporated in planar bilayer lipid membranes (BLMs) formed by various methods (Fig. 2).[31–35] In some cases, natural membranes containing ion-channels of interest are prepared by the so-called patch-clamp technique.[36,37] The use of natural receptor channels has the advantage that receptors recognize specific ligands (analytes) and hence, the sensor is highly selective to the analyte. In addition, owing to its inherent ability of amplification, the sensor possesses high sensitivity; in some cases a single receptor molecule is sufficient for generating an analytically relevant signal.

Glutamate Receptor-Based Ion-Channel Sensors for L-Glutamate. Glutamate receptor (GluR) ion-channel proteins play a key role in the neurotransmission at post-synapses in mammalian brains.[38] Upon binding of a neurotransmitter L-glutamate, GluRs open their channels; through these channels a large number of cations (Na^+ and Ca^{2+}) permeate, following their electrochemical potential gradient. We have proposed an

ion-channel sensor for L-glutamate, in which purified GluRs are incorporated into BLMs formed by the folding (Fig. 2A)[5–7] and tip-dip (Fig. 2B) methods.[4,8–10] Depending on the number of receptors in BLMs, the ion-channel sensors are classified into two groups, i.e., single- and multi-channel sensors. For constructing single-channel sensors, BLMs formed by the tip-dip method were used, since the very small area of the membranes formed at the tip ($\phi \sim 2$ μm) of a glass capillary is favorable for incorporating only one channel. On the other hand, multi-channel sensors have been constructed with membranes formed in an aperture (ϕ 100–200 μm) of a Teflon film by the Takagi–Muller–Montal folding method.

In the case of a single-channel sensor, rectangular-shaped current pulses, corresponding to the permeation of ions only through the open state of the channel, are observed. Two different approaches have been proposed for obtaining analytically relevant signals from single-channel currents, i.e., the analyses of the frequency of channel openings[4] and the integral of the single-channel current.[5,8–10] The frequency (F) is obtained from the observed mean open time (T_o) and mean closed time (T_c), as follows:

$$F = 1 / (T_o + T_c).$$

On the other hand, the integrated current, corresponding to the number of ions passed through the single-channel, is obtained by integration of the observed current with respect to time. The integrated current and frequency were found to increase with the L-glutamate concentration in the concentration ranges of 1–3 μM (M = mol dm^{-3}) and 50–500 μM, respectively. It must be emphasized that such sensitivity was achieved with a single GluR in the BLMs: the passage of more than 10^5 ions per second through the open gate generates an ionic current at the pA level that is measurable with commercially available patch-clamp apparatuses.

In the case of a multi-channel sensor, the channel current is the sum of all single-channel currents generated by GluRs in the BLMs, and is therefore much larger than a single-channel current. By using integrated multi-channel currents as an analytical signal, the sensor detects L-glutamate at the concentration level of 0.10 nM.[7] For a synthetic agonist, (2*S*,3*R*,4*S*) isomer of 2-(carboxycyclopropyl)glycine (L-CCG-IV) with the highest affinity to GluRs, the detection limit of 50 pM was achieved with the multi-channel sensor.[39]

Although only a few natural channels, including glutamate receptor ion-channels and acetylcholine receptor ion-channels, have been exploited for ion-channel sensors, the approach is generally applicable to other ligand-gated ion-channels too.

Evaluation of the Agonist Selectivity of Glutamate Receptor Ion-Channels. Among GluRs, the *N*-methyl-D-aspartic acid (NMDA) receptor plays a crucial role, for example, in brain development and memory formation.[38,40] Evaluation of the agonist selectivity in the activation of the NMDA receptor channels is important for understanding the fundamental process of neurotransmission at post-synapses and designing specific agonists. The agonist selectivity has been described in terms of binding affinity of agonists for the receptor, which is commonly reported either by the dissociation constant (and/or IC_{50}) determined with the so-called binding assay[41–43] or the EC_{50} determined with a functional assay (Fig. 3).[44–46] The dissociation constant, i.e., the reciprocal of the binding constant, is mostly evaluated based on a competitive reaction between an agonist of interest and a labeled reference agonist for the receptor, and is often reported as IC_{50} (50% inhibitory concentration of the agonist). The alternative parameter EC_{50}, the concentration of agonist required to produce 50% of the maximum response, represents the ligand affinity at physiological conditions, and is obtained from a dose–response curve, i.e., a curve for the titration of receptors with agonist. Both IC_{50} and EC_{50} are based on the amount (moles) of agonists bound to the receptor and are used as a selectivity measure that reflects the binding strength of the agonist to the receptor.

On the other hand, considering that a NMDA receptor has not only binding affinity for agonists but also the ability of ion conduction, i.e., signal transduction ability, we have proposed a new method for obtaining the agonist selectivity as the ability of signal transduction of the NMDA receptor with multi- and single-channel sensors (Figs. 3 and 4).[7–10,47] In contrast to the agonist selectivity based on the binding affinity, in our approach, the number of ions passed through channels in an open state is the measure of agonist selectivity.

(i) Multi-Channel Sensors. NMDA receptors purified from rat whole brain were incorporated in BLMs[7] and liposomes,[47] and agonist selectivities among typical agonists NM-

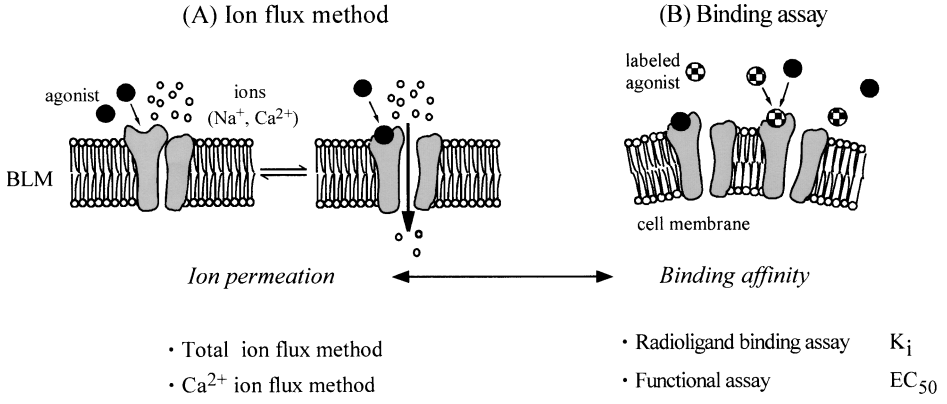

Fig. 3. Schematic representation of the approaches for evaluating agonist selectivity of the glutamate receptor ion-channel proteins. (A) Ion flux method. (B) Binding assay.

Table 1. Comparison of Ion Permeation Selectivity and Binding Affinity for the Multi-Channel Sensor

	NMDA	L-Glutamate	L-CCG-IV	Reference
Binding affinity ratio	0.022	1.0	17	42
Total ion flux method	0.47 ± 0.57	1.0	2.9 ± 1.5	7
Ca^{2+} ion flux method	0.58 ± 0.31	1.0	1.6 ± 0.4	47

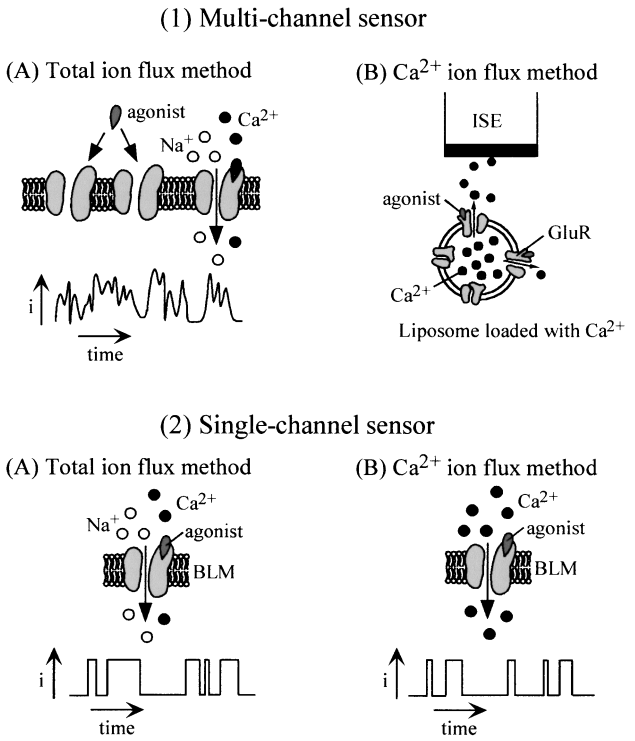

Fig. 4. Principles of evaluation of agonist selectivities based on ion fluxes with (1) multi- and (2) single-channel sensors. (A) Total ion flux method. (B) Ca^{2+} ion flux method.

DA, L-glutamate, and L-CCG-IV were evaluated based on the magnitudes of agonist-induced ion fluxes through the NMDA receptors. In the BLM sensor, integrated multi-channel currents, corresponding to the total number of ions passing through the channels in the BLMs, are measured (Fig. 4-1A). In the case of the liposome method, the amount of Ca^{2+} released from NMDA receptor-incorporated liposomes entrapping Ca^{2+} ions is determined with a Ca^{2+}-ion selective electrode in a thin-layer mode (Fig. 4-1B). Because the number of receptors in BLMs and liposomes varied from one membrane to another, the very magnitude of the agonist-induced ion fluxes was not constant and hence, did not provide an analytically relevant signal. The agonist selectivity was, therefore, evaluated as the magnitude of the ion flux induced by an agonist relative to that induced by a reference agonist. The ratio of the integrated currents thus obtained were NMDA:L-glutamate:L-CCG-IV = 0.47:1.0:2.9 for the integrated current method[7] and NMDA:L-glutamate:L-CCG-IV = 0.58:1.0:1.6 for the Ca^{2+} ion flux method (Table 1).[47] Since the brain homogenates contained different NMDA channel types (vide infra), the selectivity ratio is the average of the ion fluxes through all the channel types of NMDA receptor in rat whole brain. The range of selectivities thus obtained is much narrower than the one determined from binding affinities (NMDA:L-glutamate:L-CCG-IV = 0.022:1.0:17),[42] showing that the ability of the receptor channel to pass ions does not directly reflect the binding affinity of the agonists.

(ii) Single-Channel Sensor. Recent advances in cloning and expression of cDNAs encoding the NMDA receptors indicated that the NMDA receptors can be classified into four kinds of heteromeric channels of ζ and ε subfamily subunits, i.e., $\varepsilon1/\zeta1$, $\varepsilon2/\zeta1$, $\varepsilon3/\zeta1$, and $\varepsilon4/\zeta1$ channels.[48] Since each channel type has its own regional distribution in the brain, the $\varepsilon1$–$4/\zeta1$ channels have been assumed to play individual roles in transmembrane signaling.[38,48] It is becoming important to evaluate agonist selectivity for each channel type.

Extending the above approach to the $\varepsilon1$–$4/\zeta1$ channels, the integrated single-channel currents for the $\varepsilon1/\zeta1$, $\varepsilon2/\zeta1$, $\varepsilon3/\zeta1$, and $\varepsilon4/\zeta1$ channels in BLMs activated by NMDA, L-glutamate, and L-CCG-IV were evaluated and the magnitudes were compared for different agonists and different channel types, using single-channel sensors (Fig. 4-2A).[8,9] By incorporating a single channel in BLMs, the very magnitudes of the integrated single-channel currents of different BLMs can be compared, and therefore, the ability of each channel type to pass ions (mostly Na^+ and Ca^{2+}) is obtained directly from the magnitude of the integrated currents.

For constructing single-channel sensors, the recombinant $\varepsilon1$–$4/\zeta1$ channels were purified from Chinese hamster ovary cells expressing each channel[51] and incorporated in BLMs formed by the tip-dip method. The agonist-induced integrated single-channel currents were obtained at 50 μM agonist concentration, where the integrated currents for $\varepsilon1/\zeta1$, $\varepsilon3/\zeta1$, and $\varepsilon4/\zeta1$ channels activated by NMDA reached the saturated values and the binding sites of the $\varepsilon1$–$4/\zeta1$ receptor channels are expected, on the basis of the reported EC_{50} values (~1 μM) of the $\varepsilon1$–$4/\zeta1$ channels to L-glutamate, to be fully occupied by agonists.[44,50] Table 2 summarizes the agonist selectivity for the $\varepsilon1$–$4/\zeta1$ NMDA receptor channels based on the integrated single-channel currents. It is seen that the magnitude of the integrated single-channel current induced by the endogenous agonist L-glutamate depends on the ε-subunit composition and increases in the order of $\varepsilon2/\zeta1 > \varepsilon1/\zeta1 \approx \varepsilon4/\zeta1 > \varepsilon3/\zeta1$. On the other hand, the magnitude of the integrated currents induced by the synthetic agonists NMDA and L-CCG-IV did not differ among the four channel types, as judged from F and t tests at a confidence level of 95%. The comparison of the integrated currents for agonists for the channel type of interest (see Table 2) shows agonist selectivities in terms of ion-permeation ability. The agonist selectivity varies among the $\varepsilon1$–$4/\zeta1$ channels as follows: L-CCG-IV ≈ L-glutamate > NMDA for the $\varepsilon1/\zeta1$ and $\varepsilon4/\zeta1$ channels, L-glutamate > L-CCG-IV ≈ NMDA for the $\varepsilon2/\zeta1$ channels, and L-CCG-IV > NMDA > L-

Table 2. Comparison of Ion Permeation Selectivity and Binding Affinity for the Single-Channel Sensor

		$\varepsilon1/\zeta1$	$\varepsilon2/\zeta1$	$\varepsilon3/\zeta1$	$\varepsilon4/\zeta1$
Ion permeation	(a) Total ion flux method[a)]				
($\times10^{-13}$ C/s)	L-Glutamate	5.8 ± 0.72	7.1 ± 0.45	3.3 ± 0.21	6.0 ± 0.36
	NMDA	4.5 ± 0.55	4.0 ± 1.2	4.1 ± 0.44	3.8
	L-CCG-IV	6.6 ± 0.61	4.8 ± 1.1	5.7 ± 0.49	6.7 ± 0.92
	(b) Ca^{2+} ion flux method[b)]				
	L-Glutamate				4.6 ± 0.31
	NMDA				3.1 ± 0.21
	L-CCG-IV				5.7 ± 0.25
Binding affinity	(a) Binding constant ($1/K_i$)[c)]				
(10^6 M^{-1})	L-Glutamate	10	19	5.8	9.4
	NMDA	0.26	0.32	0.16	0.21
	(b) Apparent affinity ($1/EC_{50}$)				
	L-Glutamate[d)]	0.59	1	1	3
	NMDA[e)]	0.028	0.048	0.045	0.11

a) From Refs. 8 and 9. b) From Ref. 10. c) From Ref. 41. d) From Refs. 44 and 50. e) From Ref. 51.

glutamate for the $\varepsilon3/\zeta1$ channels. These results show that each $\varepsilon1$–$4/\zeta1$ channel has its own ability for ion permeation, i.e., its own signal transduction ability.

(iii) Signal Transduction Ability vs Binding Affinity. Several researchers have reported the binding affinities of the $\varepsilon1$–$4/\zeta1$ channels (or their rat forms NR1/NR2A-2D channels) for L-glutamate and NMDA.[41,44,50,51] To know whether the signal transduction ability of the receptor-agonist complex directly correlates with the binding strength between the receptor and agonists, we compared the agonist selectivity based on integrated single-channel currents with those based on binding affinity.

The data given in Table 2 show that the sensitivity to L-glutamate, as well as that of NMDA, for each $\varepsilon1$–$4/\zeta1$ channel, as obtained from the magnitude of integrated single-channel current, is directly correlated to the binding constants ($1/K_i$),[41] but not to the ligand affinities estimated from EC_{50} values.[44,50,51] The selectivity order for the $\varepsilon3/\zeta1$ channel based on signal transduction ability is NMDA > L-glutamate, while that based on K_i and EC_{50} values is L-glutamate > NMDA.[41,44,50,51] These results strongly indicate that the agonist selectivity in terms of signal transduction ability is not parallel to the binding one, demonstrating that evaluating the signal transduction ability of the NMDA receptor channels is important for understanding the fundamental process of the neuronal transmission.

Single-Channel Method for Evaluating Ca^{2+} Ion Flux through the $\varepsilon4/\zeta1$ Channels. Among the cations that permeate through the open pore of the NMDA receptor, Ca^{2+} works as a second messenger that regulates various kinds of intracellular signal cascades. Therefore, it is important to quantify Ca^{2+} permeation through the NMDA receptor. We have described an electrochemical method for quantifying the magnitude of Ca^{2+} ion fluxes through a single $\varepsilon4/\zeta1$ channel in BLMs and evaluated the agonist selectivity based on the very magnitude of Ca^{2+} ion fluxes (Fig. 4-2B).[10] The $\varepsilon4/\zeta1$ channel is of particular interest among the four $\varepsilon1$-$4/\zeta1$ channels, since the predominant expression of the $\varepsilon4/\zeta1$ subunit mRNA in the embryonic and early postnatal brain suggests an important role of $\varepsilon4$-containing receptors in brain development.[52] The method is based on single-channel recordings in media where Ca^{2+} is the only permeable cation. It was found that the Ca^{2+} ion current through the $\varepsilon4/\zeta1$ channel is significantly smaller (by 54%; or 27% by number of ions) than the Na^+ ion current obtained in the media where Na^+ is the only permeable cation. The integrated Ca^{2+} currents obtained are (3.1 ± 0.21) × 10^{-13} C/s for NMDA, (4.6 ± 0.31) × 10^{-13} C/s for L-glutamate, and (5.7 ± 0.25) × 10^{-13} C/s for L-CCG-IV, giving agonist selectivity based on Ca^{2+} permeation (Table 2). The magnitude of the integrated Ca^{2+} current for L-glutamate corresponds to 1.4 × 10^6 Ca^{2+} ions/s, with the relative standard deviation of 6.7%. On the basis of the precision, this method can detect a difference of 10^5 Ca^{2+} ions/s. This sensitivity is comparable to or slightly better than detection limits of methods utilizing Ca^{2+} indicators,[53,54] Ca^{2+}-ion selective electrodes,[55] and fiber optic Ca^{2+} sensors.[56] The detection limits of these methods range from 10^{-8} to 10^{-7} M Ca^{2+}, corresponding to 10^5–10^8 in the number of Ca^{2+} ions for cells having diameters of several 10 to 100 μm.

Ion-Channel Sensors Based on Gramicidin Channels. In biological membranes, there is another type of receptors, i.e., G-protein-linked receptors, which activate G-proteins upon binding with a specific ligand, leading to initiation of a chain of intracellular reactions.[57] Signaling pathways in which a G-protein directly or indirectly mediates the interaction between the receptor and a separate membrane-bound ion-channel are particularly interesting, because mimicking such indirect activation of channels initiated by the binding of a ligand (analyte) to G-protein-linked receptors provides a new principle of ion-channel sensors.

Recently, we succeeded in regulating the activity of the gramicidin channel by the binding of analytes to the receptor embedded in a planar BLM (Fig. 5A). In our approach, the BLM formed by the tip-dip method contains biotinylated phosphatidylethanolamine (biotin-PE) as a receptor and a single gramicidin channel as a molecular transducer.[58] When the receptor (biotin-PE) catches an analyte (avidin or ferritin-labeled avidin), the gramicidin monomer/dimer kinetics is modulated due to the local distortion of the BLM induced by the binding of the analyte to biotin-PE, leading to an increase in frequency of channel openings with a lifetime shorter than 100 ms. The

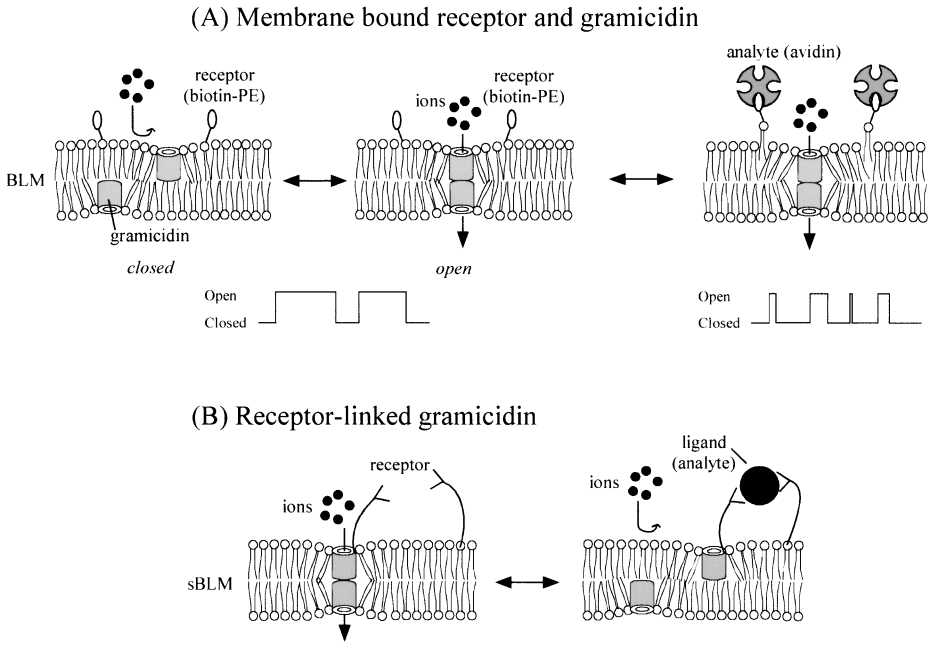

Fig. 5. Ion-channel sensor based on gramicidin channel. (A) Separate membrane receptor and gramicidin as transducer. (B) Channel-linked receptor.

frequency increased with the concentration of avidin ranging from 1×10^{-9} to 1×10^{-6} M and ferritin-labeled avidin from 1×10^{-9} to 1×10^{-8} M, respectively. The response of this sensor to ferritin-labeled avidin (molecular weight 818,000) was found to be more sensitive than that to avidin (molecular weight 68,000): the slope of the frequency vs. concentration plot was steeper for ferritin-labeled avidin than avidin. In this approach, there was no direct link between the receptor and the gramicidin channel in the bilayer membrane. The binding of an analyte to the receptor caused a modulation of the activity of the separate membrane-bound gramicidin channel. This approach has the advantage that a variety of natural and synthetic receptors having no gating mechanism can be used without directly linking them to channel molecules for designing ion-channel sensors.

Cornell et al. have reported an ion-channel switch biosensor by using gramicidin channel linked to receptors such as antibodies[20,21] and DNA[22] in a supported bilayer lipid membrane (sBLM) on a gold electrode (Fig. 5B). The channel formed by a gramicidin dimer is switched off by the binding of an analyte to the receptor site of gramicidin, resulting in a change in ion conduction (admittance). In this sensor, the gramicidin molecule had receptor sites chemically introduced for targeted analytes; hence, the approach is similar to that based on ligand-gated receptor channels.

Ion-Channel Sensors Based on Artificial Receptors

Several approaches to mimic the efficiency, selectivity, and inherent possibility for signal amplification of ligand-gated bioreceptor membranes by use of synthetic receptors were suggested.[59–63] Already in the 1970s deprotonation of acidic functional groups in self-assembled monolayers (SAMs) was shown to affect redox currents at the underlying electrodes.[64,65] However, the first demonstration of this phenomenon was only reported in the late 1980s.[12,66] Early examples of synthetic receptors used in this biomimetic approach to ion-channel sensing included anionic phospholipids,[12] macrocyclic polyamines,[66] or a cyclodextrin with ammonium groups.[66] They were deposited onto the sensor electrodes by use of the Langmuir–Blodgett (LB) method. More recently, receptors were typically self-assembled on gold electrodes by formation of covalent sulfur-gold bonds. This offers the advantage of a higher sensor stability, as will be required for real-life applications.

According to their response mechanism, two different types of ion-channel sensors based on synthetic receptors can be distinguished. On one hand, physical blocking of the *intramolecular* channels through receptor molecules by formation of inclusion-type complexes with an analyte can prevent access of redox markers to the electrode surface (Fig. 1B-2).[13,67] This approach closely mimics ion-channel proteins and seems particularly interesting for the detection of redox-inactive analytes that are electrically neutral. On the other hand, electrostatic attraction or repulsion controlled by binding of ionic analytes to the synthetic receptor layer determines how easily charged electroactive ions (often referred to as markers) are oxidized or reduced at a receptor monolayer. Most of the receptor monolayers that were used for this approach are thick enough that electron transfer across the monolayer is small. The large currents at these electrodes in the absence of repulsive interactions between the analyte and receptor monolayers result from the permeation of the electroactive markers through *intermolecular* voids (Fig. 1B-1).

As discussed fairly comprehensively in 1998,[14] a large number of sensors based on these principles were reported. Ion-channel sensors based on synthetic receptors were developed for hydrogen and metal ions, simple organic analytes such as phthalate and glucose, as well as more complex analytes such

as antibodies and concanavalin derivatives. Phosphate esters, antibiotics, oligopeptides, DNA, dendrimers, cyclodextrins, calixarenes, and antigenic groups are typical examples from the wide range of synthetic receptors that had been used in ion-channel sensors. Reports on ion-channel sensors reported in 1998 or later include sensors for estrogen,[68] inorganic cations,[69–74] pH,[75] avidin,[76] cyclic AMP,[77] DNA-binding substrates such as spermine or acridine orange,[78] phosphate,[79] protonated amines,[80] protamine,[81] heparin,[82] and oligonucleotides.[83]

In the following section of this review we will focus on the most recent trends in the field of ion-channel sensors based on synthetic receptors. The particular suitability of this type of sensor for the analysis of very hydrophilic, large analytes, will be shown with the examples of sensors for strongly hydrophilic anions and polyions, such as heparin or oligonucleotides, emphasizing the particular interfacial binding selectivity.

Ion-Channel Sensors for Hydrophilic Ions. While hydrogen bonding is central to molecular recognition in many biological and synthetic host-guest systems, complementary hydrogen bonding at phase boundaries for interfacial analyte recognition was demonstrated only recently.[84,85] Selective hydrogen bond formation at interfaces was shown by the measurement of surface pressures, FT-IR and UV-vis spectroscopy, quartz-crystal microbalances (QCM), XPS elemental analysis, and atomic force microscopy. Interestingly, hydrogen bonds to nucleotides at lipid–water interfaces were found to be stronger than in bulk water.[86] For example, 5′-ATP and 5′-AMP bind to guanidinium-functionalized monolayers 10^6–10^7 times more strongly[86] than guanidinium to phosphate in aqueous solution (1.3 M^{-1} for $H_2PO_4^-$ at pH 4; 5.1 M^{-2} for HPO_4^{2-} at pH 7).[87] A quantum-chemical model[88] and a classical electrostatic model[89] based on the Poisson–Boltzmann equation and the Debye–Hückel approximation suggest that the high stability of the interfacial complexes results from the low dielectric constant of the monolayer phase. Several examples of ion-channel sensors based on such hydrogen bond interactions have recently been reported.

We reported on the use of monolayers formed by a hydrogen bond-forming bis-thiourea receptor (Fig. 6) for channel-mimetic sensing of inorganic anions.[79] This receptor was known from our previous work[90] to bind $H_2PO_4^-$ strongly (K_{11} = 55000 M^{-1} for 1:1 binding in DMSO-d_6) and preferentially ($H_2PO_4^- > CH_3COO^- > Cl^-$) by formation of hydrogen bonds to the thiourea groups of these receptors. No type of neutral receptors that bind $H_2PO_4^-$ more strongly is known. Monolayers of this receptor were formed at the air–water interface in a Langmuir–Blodgett trough. Subsequently, a highly oriented pyrolytic graphite (HOPG) electrode was carefully brought into contact with these monolayers. Cyclic voltammetry was performed with subphase solutions containing various electroinactive analyte anions and $[Fe(CN)_6]^{4-}$ as electroactive marker. Binding of analyte anions to the receptor monolayer inhibited $[Fe(CN)_6]^{4-}$ oxidation, the influences of the analyte anions on the cyclic voltammograms being largest for HPO_4^{2-} and decreasing in the order of $HPO_4^{2-} > F^- \approx SO_4^{2-} > CH_3COO^- > Cl^-$. Interestingly, ion-selective electrodes[91] (ISEs) based on solvent polymeric membranes containing the same receptor were found to respond with a selectivity order of $Cl^- > SO_4^{2-} > CH_3COO^- > H_2PO_4^-/HPO_4^{2-}$.[92] Several of the larger anions, and in particular phosphate and sulfate, are apparently still substantially hydrated when bound to the interfacial receptor layer of the ion-channel sensor. In contrast, the selectivities of the ion-selective electrodes are based on the complete anion transfer from the aqueous into the organic membrane phase.

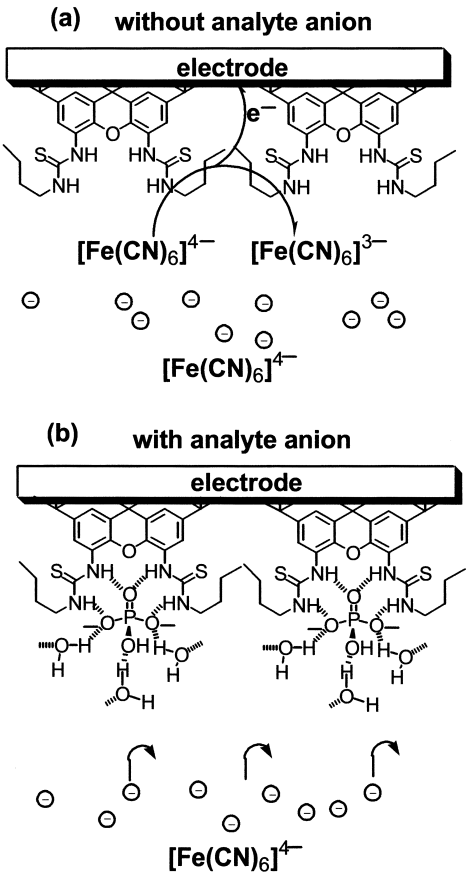

Fig. 6. Schematic illustration of $[Fe(CN)_6]^{4-}$ oxidation at the oriented monolayer of a bisthiourea receptor for the guest dihydrogen phosphate. Electrostatic repulsion between the marker and the guest hinder the oxidation of the marker.

The use of the receptor *N*, *N*′, *N*″-[benzene-1,3,5-triyltri(thiocarbonyl)]triglycine is another example for a synthetic receptor that binds to the analyte by formation of hydrogen bonds.[80] The design of this receptor was based on the goals of a stable attachment of the receptor to the gold electrode, and of minimum hindrance of electron transfer in the presence of analytes. The three thioamide groups of **1** attach the receptor to the gold electrode (Fig. 7). Reductive desorption of **1** from the electrode surface confirmed the covalent attachment of the receptor to the gold surface and showed that the receptor occupied an area of 150 Å^2 per molecule, in agreement with the expectation that this receptor adsorbs with its benzene ring parallel to the electrode surface. The protonation and deprotonation of the carboxyl groups of this receptor was investigated with $[Fe(CN)_6]^{4-}$ as marker. At low pH, the negatively charged marker can easily access the electrode surface because the receptor SAM is protonated and carries no excess charge. At

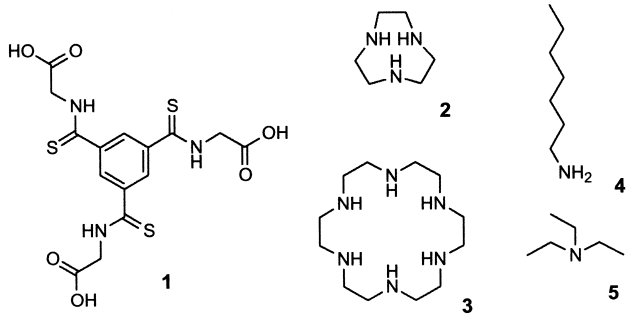

Fig. 7. Structure formulas of receptor **1** and guests **2** to **5**, shown here in electrically neutral form because several states of protonation are possible.

high pH, where the receptor SAM is deprotonated and carries an excess of negative charges, the oxidation reaction of the marker is inhibited by electrostatic repulsion between the marker and the receptor SAM. Binding of protonated amines facilitated the oxidation of $[Fe(CN)_6]^{4-}$ at high pH, showing that the protonated amines bind to the deprotonated receptors. The receptor-modified electrodes responded to 1,4,7-triazacyclononane (**2**) and 1,4,7,10,13,16-hexaazacyclooctadecane (**3**) at concentrations above 10^{-6} M and 10^{-8} M, respectively, but no responses to hexylamine (**4**) and triethylamine (**5**) were observed. The selectivity of this response is the result of the stronger binding of the protonated tri- and hexamine and reflects the larger number of charge–charge interactions and hydrogen bonds between the receptor and these two analytes.

Detection of very hydrophilic analytes with ion-channel sensors was also achieved with phosphate ester monolayers self-assembled on gold electrodes, which allowed the detection of trivalent cations down to the submicromolar concentration range with good selectivities over alkali metal ions.[74] Whereas in the absence of analyte cations the reduction of the marker $[Fe(CN)_6]^{3-}$ was hindered by electrostatic repulsion between the marker anions and phosphate groups of the receptor monolayer, binding of di- and trications to the monolayer resulted in large increases in the reduction current. The electrodes responded to trivalent cations La^{3+} and Al^{3+} approximately a hundred times more strongly than to divalent cations. For this phosphate ester receptor, the thick hydrophobic and rigid layer of alkyl that is necessary for the formation of monolayers by the Langmuir–Blodgett technique was replaced with a short alkyl spacer between the receptor group and the sulfur, which covalently binds the receptor molecule to the gold surface. This had the desired effect of facilitating the access of the marker to the electrode and seems to explain why the sensors responded to lower analyte concentrations than in case of electrodes modified with Langmuir–Blodgett monolayers of phosphate ester derivatives with long alkyl chain substituents.[12,93,94]

Ion-Channel Sensors for Protamine and Heparin. The conclusion that ion-channel sensing is particularly promising for the detection of highly hydrophilic, relatively large ions and molecules prompted us to investigate ion-channel sensors for polyions, which have high charge numbers, and are usually highly hydrophilic and large in size. Polyions were expected to bind more strongly to receptor monolayers than analytes with a small charge number, significantly affecting the access of marker ions to the electrode surface. Also, polyions are attractive analytes because they are widely applied in many application fields. For example, polysaccharides, dermatan sulfate, and iota-carrageenan are used in the food industry, polyphosphates are fertilizers, and oligonucleotides are central to biochemistry. Our interest focused first on heparin, which is used extensively as an anticoagulant in surgery and clinical chemistry, and protamine, which is used to neutralize the anticoagulant activity of heparin. While protamine is a polycation with an average charge of approximately +20, heparin is a polyanion with a typical average charge of about −70.

For the detection of protamine, gold electrodes were modified with SAMs of thioctic acid, which has two sulfur atoms that bind the receptor to the electrode.[84] Upon deprotonation, the carboxyl group provides for a negative charge that induces protamine adsorption to the electrode surface. These sensors can be used to detect protamine in concentrations as low as 0.5 μg/mL. Their response to polybrene, which is another polycation that neutralizes the anti-coagulant activity of heparin, is about a thousand times smaller than their protamine response. Because washing with 0.1 M KCl of pH 5.1 removes protamine bound to the receptor layer, the sensors can be used repeatedly. In physiological concentrations, the blood electrolytes sodium, potassium, calcium, and magnesium did not interfere. Moreover, the sensors were found to respond to protamine in diluted horse serum and were used to detect the end point in heparin–protamine titrations.

Subsequent efforts were directed at the development of ion-channel sensors for heparin itself.[82] For this purpose, the oxidation of $[Mo(CN)_8]^{4-}$ or reduction of $[Fe(CN)_6]^{3-}$ in heparin solutions was investigated with SAMs of thioctic acid that had been treated with protamine (Fig. 8). Heparin, with its multiple negative charges, neutralizes the positive charges on the protamine receptor, and at high heparin concentrations provides the electrode surface with an excess of negative charge, thereby repulsing the marker ions $[Mo(CN)_8]^{4-}$ and $[Fe(CN)_6]^{3-}$ from the electrode surface. As a result, redox currents for these negatively charge markers decrease. In a solution containing a background of inorganic ions at concentrations typical for inorganic ions in blood, a linear concentration range of 0.05–1.5 μg/mL was determined. Repeated measurements of 1.25 μg/mL heparin in a physiological ion background and in horse serum gave average heparin concentrations of 1.30 and 1.56 μg/mL, respectively. Using a fresh electrode for each horse serum sample, however, gave an average heparin concentration of 1.21 μg/mL with a standard deviation of 0.026 μg/mL. Interestingly, preliminary observations showed no observable changes in the performance of this electrode over a period of over four months during which this sensor was used for the measurement of 450 cyclic voltammograms.

Ion-Channel Sensors for Nucleotides. Kunitake and coworkers reported that monolayers with diaminotriazine and orotate head groups effectively bind thymidine and adenine, respectively.[95] These results suggested that multitopic hydrogen bonding interactions could also be used for nucleotide with ion-channel sensors. This encouraged us to investigate electrodes modified with oriented mono- or multilayers containing long alkyl chain derivatives of cytosine, thymine, or

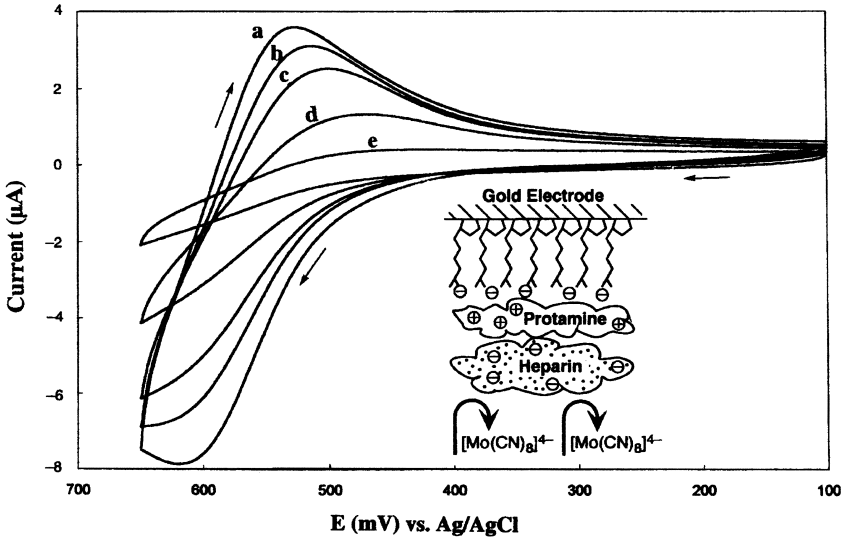

Fig. 8. Response of a heparin sensor. Heparin concentrations: (a) 0.00, (b) 1.52, (c) 2.02, (d) 2.53, and (e) 4.04 μg/mL. Scan rate 0.1 V/s.

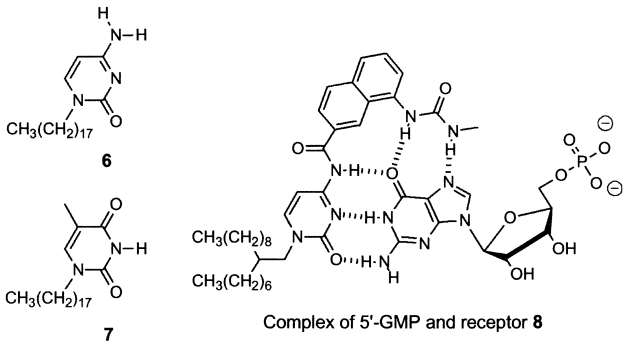

Fig. 9. Structure formulas of nucleotide receptors **6** and **7** as well as the complex of 5′-GMP with receptor **8**.

ureidonaphthylcytosine[96] (Fig. 9). Hydrogen-bond mediated complexation between the receptors and the nucleotides was used to control the access of the anionic marker $[Fe(CN)_6]^{4-}$ to the electrode surface.[97] On the one hand, a sensor based on the receptor **6** with the cytosine group that can form three hydrogen bonds to the guanine base gave a voltammetric response that was selective for guanosine 5′-monophosphate (5′-GMP) with a selectivity factor for 5′-GMP versus adenosine 5′-monophosphate (5′-AMP) of 2.38. On the other hand, a sensor based on the thymine derivative selectively responded to 5′-AMP, as would be expected for a receptor with a base pairing site for the recognition of the adenine base. The smaller selectivity of 1.89 is not surprising, because receptor-analyte interactions between the thymine moiety of receptor **7** and the adenine moiety of 5′-AMP are based on two hydrogen bonds only. The highest voltammetric discrimination of nucleotides was attained with the sensor based on octadecyl-ureidonaphthylcytosine **8**, which has besides the cytosine unit a ureidonaphthylcytosine unit, making a total of five hydrogen bonds to the analyte possible. For this case, the selectivity for 5′-GMP over 5′-AMP was 3.85.

Ion-Channel Sensors for Abasic Sites in DNA. Abasic sites are most common lesions in DNA that can be spontaneously formed under physiological conditions or by stimulation, such as with UV light and chemicals.[98,99] Electrochemical methods that have been reported for the quantification of various types of DNA damage are based on changes in redox signals of base residues in the double helix that are exposed at an electrode surface when DNAs are damaged.[100–104] These electrochemical methods are intended for detecting structural DNA damage, such as DNA strand breaks and partial unwinding of the double helix.[105]

Recently, we have designed an ion-channel sensor for abasic sites in DNA. An avidin-modified gold electrode was treated with aldehyde reactive probe (ARP) that specifically reacts with the ring-open state of abasic sites.[106] Calf thymus DNA with abasic sites was prepared by acid (pH 5)/ heat (70 °C) treatment. The cyclic voltammetric peak due to ferrocenecarboxylate ($Fc\text{-}COO^-$) as a marker at the ARP-modified electrode was found to increase with the number of abasic sites. Such changes in the peak height were not observed at an ARP-unmodified electrode and, in addition, the peak height at the ARP-modified electrode remained unchanged even when the concentration of DNA that was neither treated with acid nor heat was increased. These results strongly suggest that the ARP-modified electrode detects specifically abasic sites in DNA. The electrode is reusable after it was immersed in the solution of pH 3 for 30 min.

The permeation of the marker anion was enhanced upon binding of polyanionic DNA with abasic sites. The enhanced permeation of $Fc\text{-}COO^-$ by the binding of DNA is explained by an electrostatic repulsion between the polyanionic DNA molecules bound to ARP linked to avidin, which enlarges an intermolecular void for permeation of $Fc\text{-}COO^-$, as schematically illustrated in Fig. 10.

Detection of a One-Base Mismatch in an Oligonucleotide. The detection of specific DNA sequences is important for the diagnosis of genetic diseases. In the late 1990s, several electrochemical DNA biosensors[107,108] that rely on base-pair

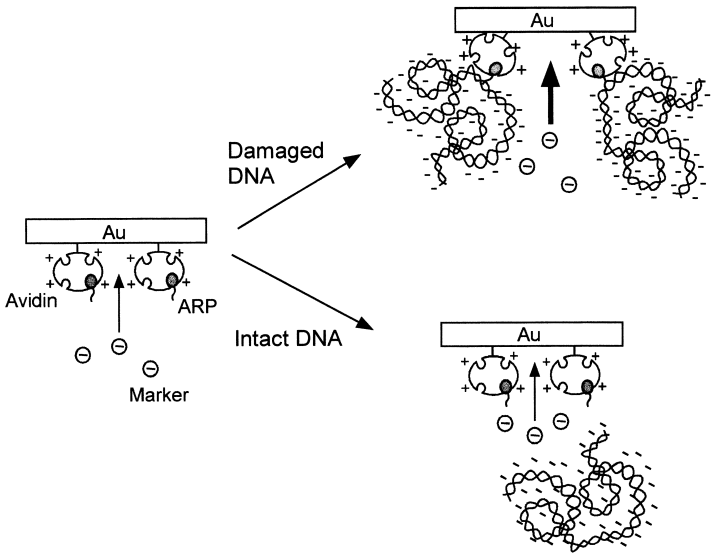

Fig. 10. Schematic representation of the possible mechanism for detecting abasic sites in DNA by an ARP-modified Au electrode.

hybridization of target DNA with oligonucleotide probes immobilized on electrodes were reported. For example, Patolsky et al. immobilized oligonucleotide probes and detected DNA using enzyme-linked Faradaic impedance spectroscopy,[109] and Takenaka et al. reported on DNA sensing with a naphthalene–ferrocene redox intercalator and an oligonucleotide probe.[110] Besides DNA, also peptide nucleic acid (PNA) was used as a probe in DNA analysis. In PNA the negatively charged phosphate backbone of DNA is replaced by a chain formed from the neutral *N*-(2-aminoethyl)glycine.[111] PNA not only highly discriminates mismatch DNA but has also a stronger binding affinity for complementary DNA than for its DNA counterpart. Wang et al. reported that PNA probes electrochemically deposited onto the surface of carbon paste electrodes show a high specificity for complementary DNA.[112] Arlinghaus et al. compared DNA and PNA probe arrays on solid supports and concluded that the discrimination of one-base mismatches is higher in the latter case.[113] DNA probes have a negatively charged backbone and binding of a complementary target oligonucleotide can at most double the surface charge on the electrode. On the contrary, PNA has a neutral backbone and binding to a complementary oligonucleotide changes to a larger extent the surface charge on the electrode, i.e., from neutral to negative. The large effect of analyte binding on the surface charge in the latter case makes the use of PNA particularly attractive for ion-channel sensing.

The detection of complementary DNA with ion-channel sensors was achieved with mixed monolayers of 6-mercapto-1-hexanol and a single strand 10-mer PNA probe linked via an ethylene glycol linker to a cysteine that covalently attaches the probe to a gold disk electrode (Fig. 11).[83] These electrodes were incubated at 47 °C in a solution of the complementary 10-mer DNA and washed with water before the electrochemical detection in solutions of the marker $[Fe(CN)_6]^{4-/3-}$. The detection limit of these sensors was in the micromolar concentration range. In contrast, the sensor did not respond to 100 μM solutions of a one-base mismatch oligonucleotide. Moreover, mismatch oligonucleotides $(dA)_{10}$ and $(dT)_{10}$ provided no responses either.

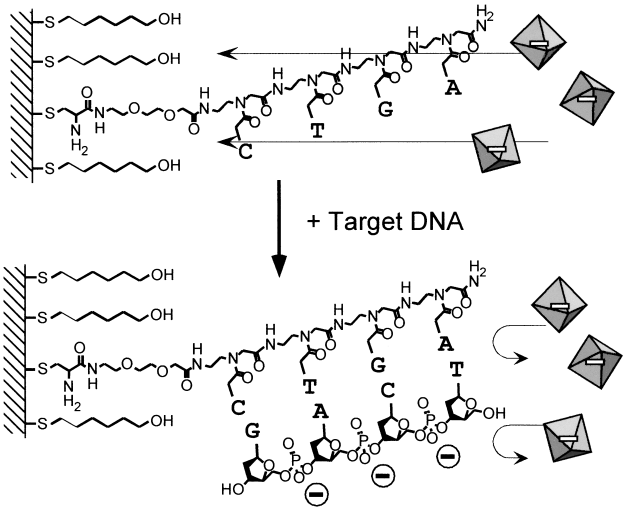

Fig. 11. Schematic of response mechanism of oligonucleotide sensor based on PNA monolayer.

Ion-Channel Sensors Based on Receptors with Intramolecular Channels. The few biomimetic intramolecular channels studied so far[13,114–121] that can be gated by recognition of chemical species are all cyclodextrin (CD) derivatives.[13,114,115] These tube-shaped rigid cyclic oligosaccharides feature largely hydrophobic internal cavities of variable size. α-, β-, and γ-CDs consist of six, seven, and eight saccharide units, respectively, resulting in diameters of the internal cavities from 0.45–0.53 and 0.60–0.70 nm for the α- and β-CDs up to 0.75 to 0.85 nm for the γ-CDs (Fig. 12). These channel-shaped CDs lend themselves to mimicking the intramolecular channels of natural transmembrane proteins.

Monolayers of an amphiphilic cyclodextrin derivative at the air–water interface in a Langmuir–Blodgett trough were used for the first example of ion-channel sensors with intramolecular receptor channel.[13] As in the case of the bisthiourea receptors for anions described above, cyclic voltammograms were

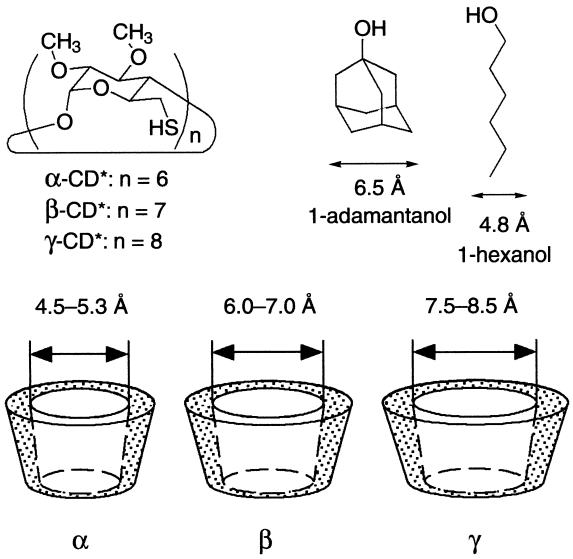

Fig. 12. Structure formulas and dimensions of cyclodextrin receptors α-, β-, and γ-CD* and their guests 1-adamantanol and 1-hexanol. The ranges for the diameters of the internal cyclodextrin cavities reflect the tapered shape of these receptors.

measured in situ upon contacting the monolayers with a planar electrode (in-trough cyclic voltammetry). Inclusion of guests into the cyclodextrin inhibited the benzoquinone reduction, apparently by sterically blocking the intramolecular channels of the cyclodextrin receptors. The modification of electrodes with SAMs of CDs[114–116,122–126] that can be used repeatedly and can withstand the contact with a real sample is an important goal on the way to real-life applications of this type of sensors. Therefore, several attempts to fabricate similar sensors with SAMs covalently attached to a metal electrode were reported recently. Osa et al. used SAMs of a CD derivative with two thiol groups per molecule.[114,115] Not a channel function but competitive adsorption of electroactive and electroinactive markers was reported for three other CD SAMs.[116,122,123]

Studies with gold electrodes modified with SAMs of CDs revealed how difficult it is to eliminate defects in CD SAMs on gold. While Osa et al. found that monothiolated β-CD SAMs are not well-packed,[114,115] Rojas et al. determined the surface coverage of perthiolated β-CD SAMs to be 64–75%.[113] On the basis of a monolayer impedance study, Galla and co-workers concluded that the weaker binding of a cyclodextrinmonothiol through only one thiol[124–126] allows the reconformation of the monolayer after chemisorption, improving the packing density of the CD SAM.

Unfortunately, the reaction of electroactive markers at SAM defects results in background currents that cannot be controlled by the guest. In particular, background currents are large if the redox reaction rate of the redox marker at a SAM defect is larger than in the cyclodextrin cavity. As a result, ion-channel sensors with intramolecular channels require very well packed SAMs. For this purpose, we investigated SAMs of thiolated CDs (called hereafter α-, β-, and γ-CD*; see Fig. 12) on hanging mercury drop electrodes (HMDEs).[127,128] The mercury surface is extremely flat and because of the liquid nature of mercury is more conducive to lateral movements of the CD molecules than solid surfaces such as gold. Indeed, mean molecular areas of the β- and γ-CD* in SAMs on HMDEs, as evaluated by reductive cleavage of the Hg–S bonds, agreed with those of the corresponding monolayers at the air–water interface at the lateral pressure 4.0 mN m^{-1} within 5%, showing that self-assembly at the surface of HMDEs provides well-packed CD monolayer. The redox reaction of $[Ru(NH_3)_6]^{3+}$ at these SAMs confirmed the high packing density of these SAMs.

The recognition of electrically charged and neutral guests at CD* SAMs on HMDEs was studied by the measurement of interfacial capacitances.[127] Whereas the hydrophobic interactions are the major driving force in size-selective binding of hydrophobic molecules, measurement of the interfacial capacitance showed that electrostatic double-layer forces are primarily responsible for selective inclusion of small hydrophilic anions into CD* SAMs. In the range of potentials where the electrode is positively charged, the interfacial capacitance depends on the type of electrolyte anions and on the applied potential. Whereas the smaller and less well solvated anions Cl^-, NO_3^-, and ClO_4^- are included into the cyclodextrin cavities of these monolayers, the larger and more strongly solvated anions F^-, SO_4^{2-}, and $H_2PO_4^-$ are excluded. The potential dependence of the inclusion constants shows that electrical double layer forces are the driving forces for NO_3^- inclusion. Interestingly, competitive binding of hydrophobic guest molecules decreases the interfacial capacitance by excluding the anions. Fitting Langmuir isotherms to the plots of the interfacial capacitance as a function of the guest concentration yielded the binding constants of 1.0×10^4 M^{-1} and 2.6×10^4 M^{-1} for the β- and γ-CD* SAMs, respectively. Binding of adamantanol to α-CD* SAMs could not be observed, apparently because this guest is too large for the internal cavity of the α-CD*. In contrast, 1-hexanol binds to α-CD* SAM with a binding constant of 8.9×10^4 M^{-1}.

Subsequently, the redox reaction of benzoquinone at CD* SAMs was investigated in the absence and presence of neutral guest molecules.[128] In the case of the β- and γ-CD* SAMs, the electron transfer through the intramolecular channels was blocked by 1-adamantanol as a guest. In consistency with the observation that 1-adamantanol does not bind to α-CD*, this guest had no effect on the reduction of benzoquinone at α-CD* SAMs. This confirms the size-selective binding of 1-adamantanol to the β- and γ-CD* SAMs.

Conclusions

In this review, approaches to chemical sensing with receptor-modified electrodes that mimic the function of natural membranes containing biological and synthetic receptor channels were discussed. While the first demonstrations of channel-mimicking sensors modified with synthetic receptors were performed with LB mono- and multilayers, the current trend is to use SAM-modified electrodes, which offer the advantage of a higher membrane stability and are more promising for routine applications. However, in-trough voltammetry is still of interest if receptors without thiol groups that can be used for self-assembly are tested as an evaluation of new receptor classes. Higher selectivities by improved receptor design, taking

into account the particular advantages of interfacial analyte recognition, are going to be important aspects of the future research and application of ion-channel-mimetic sensors based on synthetic receptors. Ion-channel sensors for antibodies, protamine, heparin, abasic DNA sites, and oligonucleotides appear to represent important steps in this direction. Although the stability of biological receptors is less than that of artificial ones, biological receptors have some potential as sensing elements for ion-channel sensors. The sensitivity and selectivity of biological receptors are often excellent, and in addition, biological systems provide ideas for new principles of chemical sensors. Because of an increasing interest in detecting bioactive substances not only in vitro but also in vivo, for example, in single cells, brain slices and brains, the design of in situ membrane sensors is the important area of future studies.

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References

1 L. Stryer, "Biochemistry," 4th ed, Freeman, New York (1995), pp. 291–309, 341–347.

2 "Interfacial Design and Chemical Sensing," ed by T. E. Mallouk and D. J. Harrison, American Chemical Society, Washington, D.C. (1994).

3 M. Sugawara, A. Hirano, and Y. Umezawa, "Affinity Biosensors, Techniques and Protocols," ed by K. R. Rogers and A. Mulchandani, Humana Press, Totowa (1998), p. 209.

4 M. Uto, E. K. Michaelis, I. F. Hu, Y. Umezawa, and T. Kuwana, *Anal. Sci.*, **6**, 221 (1990).

5 H. Minami, M. Sugawara, K. Odashima, Y. Umezawa, M. Uto, E. K. Michaelis, and T. Kuwana, *Anal. Chem.*, **63**, 2787 (1991).

6 H. Minami, M. Uto, M. Sugawara, K. Odashima, Y. Umezawa, E. K. Michaelis, and T. Kuwana, *Anal. Sci.*, **7**, 1675 (1991).

7 M. Sugawara, A. Hirano, M. Rehák, J. Nakanishi, K. Kawai, H. Sato, and Y. Umezawa, *Biosens. Bioelectron.*, **12**, 425 (1997).

8 A. Hirano, M. Sugawara, Y. Umezawa, S. Uchino, and S. Nakajima-Iijima, *Biosens. Bioelectron*, **15**, 173 (2000).

9 A. Hirano, M. Wakabayashi, M. Sugawara, S. Uchino, and S. Nakajima-Iijima, *Anal. Biochem.*, **283**, 258 (2000).

10 M. Wakabayashi, A. Hirano, M. Sugawara, S. Uchino, and S. Nakajima-Iijima, *J. Pharm. Biomed. Anal.*, **24**, 453 (2001).

11 S. R. Reiken, B. J. Van Wie, H. Sutisna, D. F. Moffett, A. R. Koch, M. Silber, and W. C. Davis, *Biosens. Bioelectron.*, **11**, 91 (1996).

12 M. Sugawara, K. Kojima, H. Sazawa, and Y. Umezawa, *Anal. Chem.*, **59**, 2842 (1987).

13 K. Odashima, M. Kotato, M. Sugawara, and Y. Umezawa, *Anal. Chem.*, **65**, 927 (1993).

14 P. Bühlmann, H. Aoki, K. P. Xiao, S. Amemiya, K. Tohda, and Y. Umezawa, *Electroanalysis*, **10**, 1149 (1998), and references therein.

15 H. Sato, H. Hakamada, Y. Yamazaki, M. Uto, M. Sugawara, and Y. Umezawa, *Biosens. Bioelectron.*, **13**, 1035 (1998).

16 Y. Adachi, M. Sugawara, K. Taniguchi, and Y. Umezawa, *Anal. Chim. Acta*, **281**, 577 (1993).

17 N. Sugao, M. Sugawara, H. Minami, M. Uto, and Y. Umezawa, *Anal. Chem.*, **65**, 363 (1993).

18 M. Uto, H. Yoshida, M. Sugawara, and Y. Umezawa, *Anal. Chem.*, **58**, 1798 (1986).

19 M. Sugawara, M. Omoto, H. Yoshida, and Y. Umezawa, *Anal. Chem.*, **60**, 2301 (1988).

20 B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek, and R. J. Pace, *Nature*, **387**, 580 (1997).

21 B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek, and R. J. Pace, Novartis Found. Symp., **225**, 231 (1999).

22 S. W. Lucas and M. M. Harding, *Anal. Biochem.*, **282**, 70 (2000).

23 M. Sugawara, S. B. Khoo, S. Yoshiyagawa, K. Yagi, H. Sato, M. Namba, M. Wakabayashi, H. Minami, H. Sazawa, K. Odashima, and Y. Umezawa, *Anal. Sci.*, **10**, 343 (1994).

24 H. Minami, N. Sato, M. Sugawara, and Y. Umezawa, *Anal. Sci.*, **7**, 853 (1991).

25 H. Sato, M. Wakabayashi, T. Ito, M. Sugawara, and Y. Umezawa, *Anal. Sci.*, **13**, 437 (1997).

26 M. Maeda, Y. Mitsuhashi, K. Nakano, and M. Takagi, *Anal. Sci.*, **8**, 83, (1992).

27 M. Maeda, K. Nakano, S. Uchida, and M. Takagi, *Chem. Lett.*, **1994**, 1805.

28 S. S. Babkina, E. P. Medyantseva, H. C. Budnikov, and M. P. Tyshlek, *Anal. Chem.*, **68**, 3827 (1996).

29 A. Bardea, P. Patolsky, A. Dagan, and I. Willner, *J. Chem. Soc., Chem. Commun.*, **1999**, 21.

30 Y. Katayama, Y. Ohuchi, M. Nakayama, M. Maeda, H. Higashi, and Y. Kudo, *Chem. Lett.*, **1997**, 883.

31 P. Mueller, D. O. Rudin, H. T. Tien, and W. C. Wescott, *Nature*, **194**, 979 (1962).

32 P. Mueller, D. O. Rudin, H. T. Tien, and W. C. Wescott, *J. Phys. Chem.*, **67**, 534 (1963).

33 M. Montal and P. Mueller, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 3561 (1972).

34 M. Takagi, K. Azuma, and U. Kishimoto, *Annu. Rep. Biol. Works Fac. Sci. Osaka Univ.*, **13**, 107 (1965).

35 R. Coronado and R. Latorre, *Biophys. J.*, **43**, 231 (1983).

36 E. Neher and B. Sakmann, *Nature*, **260**, 799 (1976).

37 O. P. Hamill, A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth, *Pflügers Arch.*, **391**, 85 (1981).

38 R. Dingledine, K. Borges, D. Bowie, and S. F. Traynelis, *Pharmacol. Rev.*, **51**, 7 (1999).

39 M. Sugawara and A. Hirano, *Bunseki Kagaku* (in Japanese), **47**, 903 (1998).

40 T. V. P. Bliss and G. L. Collingridge, *Nature*, **361**, 31 (1993).

41 D. J. Laurie and P. H. Seeburg, *Eur. J. Pharmacol.*, **268**, 335 (1994).

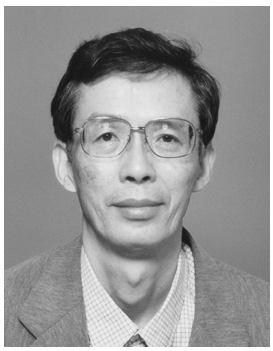
42 M. Kawai, Y. Horikawa, T. Ishihara, K. Shimamoto, and Y. Ohfune, *Eur. J. Pharmacol.*, **211**, 195 (1992).

43 H. J. Olverman, A. W. Jones, K. N. Mewett, and J. C. Watkins, *Neuroscience*, **26**, 17 (1988).

44 T. Kutsuwada, N. Kashiwabuchi, H. Mori, K. Sakimura, E. Kushiya, K. Araki, H. Meguro, H. Masaki, T. Kumanishi, M.

Arakawa, and M. Mishina, *Nature*, **358**, 36 (1992).
45 T. Priestley, P. Laughton, J. Myers, B. Le Bourdellés, J. Kerby, and P. J. Whiting, *Mol. Pharmacol.*, **48**, 841 (1995).
46 M. A. Varney, C. Jachec, C. Deal, S. D. Hess, L. P. Daggett, R. Skvoretz, M. Urcan, J. H. Morrison, T. Moran, E. C. Johnson, and G. Veliçelebi, *J. Pharmacol. Exp. Ther.*, **279**, 367 (1996).
47 H. Radecka, J. Nakanishi, A. Hirano, M. Sugawara, and Y. Umezawa, *J. Pharm. Biomed. Anal.*, **19**, 205 (1999).
48 H. Mori and M. Mishina, *Neuropharmacology*, **34**, 1219 (1995).
49 S. Uchino, Y. Kudo, W. Watanabe, S. Nakajima-Iijima, and M. Mishina, *Mol. Brain. Res.*, **44**, 1 (1997).
50 K. Ikeda, M. Nagasawa, H. Mori, K. Araki, K. Sakimura, M. Watanabe, Y. Inoue, and M. Mishina, *FEBS Lett.*, **313**, 34 (1992).
51 A. L. Buller and D. T. Monaghan, *Eur. J. Pharmacol.*, **320**, 87 (1997).
52 M. Watanabe, Y. Inoue, K. Sakimura, and M. Mishina, *Neuro Report*, **3**, 1138 (1992).
53 A. Miyawaki, J. Llopis, R. Heim, J. M. McCaffery, J. A. Adams, M. Ikura, and R. Y. Tsien, *Nature*, **388**, 882 (1997).
54 R. Y. Tsien, *Biochemistry*, **19**, 2396 (1980).
55 S. Shalom, A. Strinkovski, G. Peleg, S. Druckmann, A. Krauss, A. Lewis, M. Linial, and M. Ottolenghi, *Anal. Biochem.*, **244**, 256 (1997).
56 W. Tan, R. Kopelman, S. L. R. Barker, and M. T. Miller, *Anal. Chem.*, **71**, 606A (1999).
57 B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, "Molecular Biology of the Cell," 2nd ed, Garland Publishing, New York (1989), pp. 697–699.
58 A. Hirano, M. Wakabayashi, Y. Matsuno, and M. Sugawara, submitted.
59 K. Odashima, M. Sugawara, and Y. Umezawa, *Trends Anal. Chem.*, **10**, 207 (1991).
60 K. Odashima, M. Sugawara, and Y. Umezawa in "Interfacial Design and Chemical Sensing, ACS Symp. Ser. 561," ed by T. E. Mallouk and J. D. Harrison, American Chemical Society, Washington, DC (1994), Chap. 11.
61 K. Odashima, P. Bühlmann, M. Sugawara, K. Tohda, K. Koga, and Y. Umezawa, in "Advances in Supramolecular Chemistry," ed by G. W. Gokel, JAI Press Inc., Greenwich, CT (1997), Vol. 4, p. 211.
62 J. L. Sessler, P. I. Sansom, A. Andrievsky, and V. Kral, in "Supramolecular Chemistry of Anions," ed by A. Bianchi, K. Bowman-James, and E. Garcia-España, Wiley-VCH, New York (1997), p. 355.
63 H. T. Tien, R. H. Barish, L.-Q. Gu, and A. L. Ottova, *Anal. Sci.*, **14**, 3 (1998).
64 R. F. Lane and A. T. Hubbard, *J. Phys. Chem.*, **77**, 1411 (1973).
65 P. R. Moses, L. Wier, and R. W. Murray, *Anal. Chem.*, **47**, 1882 (1975).
66 S. Nagase, M. Kataoka, R. Naganawa, R. Komatsu, K. Odashima, and Y. Umezawa, *Anal. Chem.*, **62**, 1252 (1990).
67 W. Kutner, J. Wang, M. L'Her, and R. P. Buck, *Pure Appl. Chem.*, **70**, 1302 (1998).
68 M. Murata, M. Nakayama, H. Irie, K. Yakabe, K. Fukuma, Y. Katayama, and M. Maeda, *Anal. Sci.*, **17**, 387 (2001).
69 Y. Miura, S. Kimura, S. Kobayashi, Y. Imanishi, and J. Umemura, *Biopolymers*, **55**, 391 (2000).
70 T. Ito, *J. Electroanal. Chem.*, **495**, 87 (2001).
71 S. Flink, H. Schonherr, G. J. Vansco, G. A. J. Geurts, K. G. C. van Leerdam, F. C. J. M. van Veggel, and D. N. Reinhoudt, *J. Chem. Soc., Perkin Trans. 2*, **2141** (2000).
72 K. Bandyopadhyay, H. Y. Liu, S. G. Liu, and L. Echegoyen, *Chem. Commun.*, **141** (2000).
73 P. Diao, D. L. Jiang, X. L. Cui, D. P. Gu, R. T. Tong, and B. Zhong, *Bioelectrochem. Bioenerg.*, **45**, 173 (1998).
74 M. Takaya, P. Bühlmann, and Y. Umezawa, *Mikrochim. Acta*, **132**, 55 (1999).
75 L. Q. Luo, Z. L. Cheng, X. R. Yang, and E. K. Wang, *Chin. J. Chem.*, **18**, 863 (2000).
76 H. Kuramitz, K. Sugawara, and S. Tanaka, *Electroanalysis*, **12**, 1299 (2000).
77 Y. Katayama, Y. Ohuchi, H. Higashi, Y. Kudo, and M. Maeda, *Anal. Chem.*, **72**, 4671 (2000).
78 K. Nakano, S. Uchida, Y. Mitsuhashi, Y. Fujita, H. Taira, and M. Maeda, *Polym. Sens.*, **690**, 34 (1998).
79 K. P. Xiao, P. Bühlmann, and Y. Umezawa, *Anal. Chem.*, **71**, 1183 (1999).
80 H. Aoki, P. Bühlmann, and Y. Umezawa, *J. Electroanal. Chem.*, **473**, 105 (1999).
81 V. P. Y. Gadzekpo, K. P. Xiao, H. Aoki, P. Bühlmann, and Y. Umezawa, *Anal. Chem.*, **71**, 5109 (1999).
82 V. P. Y. Gadzekpo, P. Bühlmann, K. P. Xiao, H. Aoki, and Y. Umezawa, *Anal. Chim. Acta*, **411**, 163 (2000).
83 H. Aoki, P. Bühlmann, and Y. Umezawa, *Electroanalysis*, **12**, 1272 (2000).
84 C. M. Paleos and D. Tsiourvas, *Adv. Mater.*, **9**, 695 (1997).
85 K. Kurihara, *Colloids Surf.*, **123–124**, 425 (1997).
86 K. Ariga and T. Kunitake, *Acc. Chem. Res.*, **31**, 371 (1998).
87 B. Springs and P. Haake, *Bioorg. Chem.*, **6**, 181 (1977).
88 M. Sakurai, H. Tamagawa, Y. Inoue, K. Ariga, and T. Kunitake, *J. Phys. Chem. B*, **101**, 4810 (1997).
89 H. Tamagawa, M. Sakurai, Y. Inoue, K. Ariga, and T. Kunitake, *J. Phys. Chem. B*, **101**, 4817 (1997).
90 P. Bühlmann, S. Nishizawa, K. P. Xiao, and Y. Umezawa, *Tetrahedron*, **53**, 1647 (1997).
91 E. Bakker, P. Bühlmann, and E. Pretsch, *Chem. Rev.*, **97**, 3083 (1997).
92 K. P. Xiao, P. Bühlmann, S. Nishizawa, S. Amemiya, and Y. Umezawa, *Anal. Chem.*, **69**, 1038 (1997).
93 N. Nakashima and T. Taguchi, in "Interfacial Design and Chemical Sensing. ACS Symp. Ser. 561," ed by T. E. Mallouk and J. D. Harrison, American Chemical Society, Washington, DC (1994), Chap. 13.
94 N. Nakashima and T. Taguchi, *Colloids Surf.*, **103**, 159 (1995).
95 K. Taguchi, K. Ariga, and T. Kunitake, *Chem. Lett.*, **1995**, 701, and references therein
96 S. Amemiya, P. Bühlmann, and Y. Umezawa, *Chem. Commun.*, **1997**, 1027.
97 K. Tohda, S. Amemiya, T. Ohki, S. Nagahora, S. Tanaka, P. Bühlmann, and Y. Umezawa, *Isr. J. Chem.*, **37**, 267 (1997).
98 L. A. Loeb and B. D. Preston, *Annu. Rev. Genet.*, **20**, 201 (1986).
99 T. Lindahl and B. Nyberg, *Biochemistry*, **11**, 3610 (1972).
100 M. Fojta, V. Stanková, E. Palecek, P. Koscielniak, and J. Mitás, *Talanta*, **46**, 155 (1998).
101 M. Fojta and E. Palecek, *Anal. Chim. Acta*, **342**, 1 (1997).
102 X. Cai, G. Rivas, P. A. M. Farias, H. Shiraishi, J. Wang, M. Fojta, and E. Palecek, *Bioelectrochem. Bioenerg.*, **40**, 41 (1996).
103 J. Wang, G. Rivas, M. Ozsoz, D. H. Grant, X. Cai, and C. Parrado, *Anal. Chem.*, **69**, 1457 (1997).

104 J. Mbindyo, L. Zhou, Z. Zhang, J. D. Stuart, and J. F. Rusling, *Anal. Chem.*, **72**, 2059 (2000).
105 E. Palecek, M. Fojta, M. Tomschik, and J. Wang, *Biosens. Bioelectron.*, **13**, 621 (1998).
106 N. Shimokawa, A. Hirano, and M. Sugawara, submitted.
107 J. Wang, *Chem. Eur. J.*, **5**, 1681 (1999).
108 J. Wang, *Anal. Chem.*, **71**, 328R (1999).
109 F. Patolsky, E. Katz, A. Bardea, and I. Willner, *Langmuir*, **15**, 3703 (1999).
110 S. Takenaka, K. Yamashita, M. Takagi, Y. Uto, and H. Kondo, *Anal. Chem.*, **72**, 1334 (2000).
111 P. E. Nielsen, M. Egholm, R. H. Berg, and O. Buchardt, *Science*, **254**, 1497 (1991).
112 J. Wang, E. Palecek, P. E. Nielsen, G. Rivas, X. Cai, H. Shiraishi, N. Dontha, D. Luo, and P. A. M. Farias, *J. Am. Chem. Soc.*, **118**, 7667 (1996).
113 H. F. Arkinghaus, M. N. Kwoka, and K. B. Jacobson, *Anal. Chem.*, **69**, 3747 (1997).
114 P. He, J. Ye, Y. Fang, I. Suzuki, and T. Osa, *Anal. Chim. Acta*, **337**, 217 (1997).
115 P. He, J. Ye, Y. Fang, I. Suzuki, and T. Osa, *Electroanalysis*, **9**, 68 (1997).
116 M. Weisser, G. Nelles, G. Wenz, and S. Mittler-Neher, *Sens. Actuators, B*, **38**–**39**, 58 (1997).
117 T. M. Fyles and W. F. van Straaten-Nijenhuis, in "Comprehensive Supramolecular Chemistry," ed by D. N. Reinhoudt, Pergamon, Oxford (1996), Vol. 10, p. 53.
118 K. S. Akerfeldt, P. K. Kienker, J. D. Lear, and W. F. DeGrado, in "Comprehensive Supramolecular Chemistry," ed by D. N. Reinhoudt, Pergamon, Oxford (1996), Vol. 10, p. 659.
119 K. Motesharei and M. R. Ghadiri, *J. Am. Chem. Soc.*, **119**, 11306 (1997).
120 H. Kitano, Y. Taira, and H. Yamamoto, *Anal. Chem.*, **72**, 2976 (2000).
121 I. Suzuki, K. Murakami, J. Anzai, T. Osa, P. G. He, and Y. Z. Fang, *Talanta*, **47**, 487 (1998).
122 Y. Maeda, T. Fukuda, H. Yamamoto, and H. Kitano, *Langmuir*, **13**, 4187 (1997).
123 M. T. Rojas, R. Koniger, J. F. Stoddart, and A. E. Kaifer, *J. Am. Chem. Soc.*, **117**, 336 (1995).
124 C. Henke, C. Steinem, A. Janshoff, G. Steffan, H. Luftmann, M. Sieber, and H.-J. Galla, *Anal. Chem.*, **68**, 3158 (1996).
125 A. Michalke, A. Janshoff, C. Steinem, C. Henke, M. Sieber, and H.-J. Galla, *Anal. Chem.*, **71**, 2528 (1999).
126 A. Janshoff, C. Steinem, A. Michalke, C. Henke, and H.-J. Galla, *Sens. Actuators B*, **70**, 243 (2000).
127 R. V. Chamberlain, II, K. Slowinska, M. Majda, P. Bühlmann, H. Aoki, and Y. Umezawa, *Langmuir*, **16**, 1388 (2000).
128 H. Aoki, T. Ohki, P. Bühlmann, and Y. Umezawa, manuscript in preparation.



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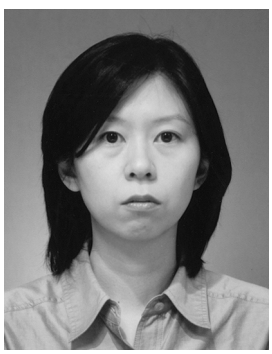

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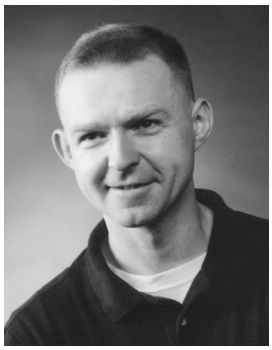

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