Biosensor development
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Current biosensor developments can be summarised by different trends. For traditional enzymatic biosensors such as glucose sensors, steady improvements of well known basic principles have been made in order to achieve better sensor stability. On the other hand, new affinity sensors such as nucleic acid sensors, transmembrane sensors, and sensors utilising whole cells or even cell networks have become of increasing interest. New ways to miniaturise biosensors and to control their interfaces down to the molecular level have been introduced (the bioelectronics approach). High-throughput screening based on various signal transduction principles has become of increasing importance.

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Abbreviations
AFM atomic force microscope/microscopy
PNA peptide nucleic acid

Introduction
Chemical sensing is a process by which selected information about chemical composition is obtained in real time. The principle is shown in Figure 1. The main part of a typical sensor consists of a chemically sensitive layer coupled to a transducer that converts the (bio)chemical change into a signal, which is then usually translated into a digital electronic result. Typical transducers are summarised in Figure 2.

A biosensor is a particular type of chemical sensor that uses the recognition properties of biological components in the sensitive layer. The majority of current biosensors may be classified by four different principles: bioaffinity, catalytic, transmembrane and cell sensors. The active part of most of the transmembrane and cell sensors is usually also based on affinity or catalytic principles.

Current research and development in bioanalytical probing aims at improved stability, selectivity and sensitivity of biosensing devices. This requires the control of physico-chemical properties of their different interfaces under the various application conditions; control of the physico-chemical properties of a biosensor device is a common bottleneck in the development of all next generation biosensors because of their insufficient stability and reproducibility of their interface [1,2]. (Biosensors have an interface between the biological (chemically sensitive) layer and the transducer substrate, and where the biological layer surface is touched by the analyte solution.)

There is an increasing trend towards miniaturisation of spectrometers, chromatographs and detectors which are traditionally used in bioanalytical chemistry. This requires adaptation of microfabrication and nanofabrication techniques. As a result of this development, a clear distinction between miniaturised instruments and ‘real’ biosensors becomes increasingly difficult to determine and, in certain cases, meaningless. This statement holds particularly true for recent trends in the development of optical and electroanalytical biosensors. In addition, the practical application of any sensor or analytical instrument requires the use of complete analysis systems and hence the development and optimisation of the same types of components for sample handling, pumps, filters, membranes and

Figure 1
Schematic setup of a (bio)chemical sensor. The species to be detected pass through a filter and hit the chemically sensitive layer. The interaction between the analyte molecules and this layer causes a change in the physico-chemical properties of the layer (e.g. changed mass, optical properties, and so on; see Figure 3). These changed properties are converted by the transducer to an electronic signal, which can be analysed.
sample conditioning (e.g. enrichment, conversion by catalysts or enzymes, and so on).

The biochemical composition of complex mixtures may be characterised in selected cases by using arrays of biosensors. In these cases, the data from the individual sensors are analysed by suitable pattern recognition methods, which leads to a biochemical analysis result.

In this review we summarise current improvements and trends in biosensor development. An overview of the state-of-the-art was presented at the Fifth World Congress on Biosensors held in June 1998 in Berlin, Germany. Highlights of this conference, which consisted of invited plenary and keynote lectures, as well as some general trends, will be summarised briefly.

Selected highlights in biosensor development

Bioaffinity sensors

Bioaffinity sensors comprise a group of highly versatile biosensors. In recent years, DNA probes in particular, and nucleic acid sensors in general, have gained increasing importance. The development of DNA hybridisation biosensors holds great promise for obtaining sequence-
Different types of bioaffinity (or immuno) sensors with direct signal transduction in the absence of any labelling of antigens or antibodies. (a) Mass-sensitive detection (e.g. by a quartz crystal microbalance) mass changes, $\Delta m$, lead to frequency changes, $\Delta f$. (b) Measurements of electrochemical impedance or related electrochemical properties (changes of capacitance, $\Delta C$, of resistance, $\Delta R$, etc., by measurements of currents, $I$, and voltages, $U$). (c) Grating couplers (intensity changes from $I_0$ of the incident light beam [arrow] before the interaction with the analyte to the intensity $I$ after the interaction and phase changes, $\Delta \phi$, for different changes in optical thickness [i.e. in refractive index, $n$, and film thickness, $d$]). (d) Surface plasmon resonance. (The graph on the right hand shows typical measurement curves before and after analyte interaction.) (e) Reflectometric interference (intensity changes for different wavelengths, $\lambda$.) The graph on the right hand shows typical measurement curves before and after analyte interaction. (f) Mach-Zehnder interferometer (intensity changes, $\Delta I$, and phase changes, $\Delta \phi$ are measured). A, analyte; R, immobilised recognition units.

New applications for antigen–antibody and enzyme–enzyme inhibitor sensors in the field of medical diagnostics will be seen in the next few years. Mass-sensitive detection by a quartz crystal microbalance (see Figure 3a) is becoming of increasing importance in these applications because this method is label-free and does not require optically transparent analyte solutions, and hence may be used with whole blood or urine.

Another trend concerns application of bioaffinity sensors in the field of high-throughput screening, in particular in the pharmaceutical industries. For this purpose, miniaturised, fast and cheap detection methods have to be applied. Optical principles with suitable labels are often used today but other transducer principles, in particular those that are label-free, may become of major importance during the next few years.

New applications can also be found in environmental control. Selective and sensitive detection of various heavy metals ($\text{Cu}^{2+}$, $\text{Cd}^{2+}$, $\text{Hg}^{2+}$ and $\text{Zn}^{2+}$) at low concentrations is extremely important for environmental protection. Biosensors have been designed that are based on bioengineered proteins. The biosensor monitors conformational
changes caused by the binding of the metal ion to the engineered protein, and thus allows heavy metal detection in the femtomolar range. In this context, gold electrodes have been used as transducers, onto which monolayers of thiol groups were self-assembled to which the protein was bound with a suitable cross-linking agent. Two differently engineered proteins (glutathione-S-transferase-SmtA and MerR), displaying different selectivity for heavy metals, were used for biosensor development [4]. The conformational change resulting from the binding of the metal ion to the engineered protein causes a change in the capacitance which is proportional to the concentration of the metal ions to be determined [4].

A completely different application for bioaffinity sensors concerns biological warfare. There is an increasing awareness and concern for the vulnerability of the civilian population to a biologically directed terrorist attack. Indeed, the ability to accomplish such an attack does not require the use of sophisticated technology or large amounts of biological agent; however, detecting such an attack with current technology requires large amounts of the biological agent and could take a disastrously long period of time. (An environment containing one particle of Bacillus anthracis per litre of air can distribute a lethal dose in 40 minutes of normal respiration.) This places a premium on the sensing performance parameters — speed and sensitivity. Fortunately, there has been significant recent progress in developing sensing modalities for a wide range of pathogens. Many of the more efficacious sensing schemes employ optics and biomolecules in their design [5].

Another trend concerns the use of biomimetic recognition sites instead of biological units. Compared with antibodies, enzymes or other biological receptors, such materials possess inherent advantages including chemical, thermal and mechanical stability, low cost, ease of handling and utility in situations where biomolecules are not available (for example, where receptors have not been identified, are too unstable or too difficult to keep in a technical surrounding). It may also be possible to tailor the properties of these biomimetic recognition sites, providing further advantages over biological units.

In the context of biomimetic recognition sites, libraries of compounds are not only of interest as analytes, but can also be used as coatings for sensor arrays. Cyclopeptides are a promising class of macrocyclic host molecules, a large variety of which can be synthesised by coupling amino acids first to give linear peptides that are subsequently cyclised. With the principles of combinatorial chemistry, approximately 250 commercially available building blocks can be utilised for the synthesis of linear peptides and, accordingly, 250^6 = 2.44 × 10^14 cyclohexapeptides can be synthesised. This number can be further increased with specially designed and prepared monomeric building blocks. With such cyclic peptides, amino acids could be detected in water by coupling the peptides to a quartz crystal microbalance (see Figure 3) and detecting the mass change upon amino acid interaction (W Göpel and co-workers, unpublished data). In another example, 266,144 hexapeptides were used as affinity ligands for glycosylated haemoglobin [6].

Another technique that is used in conjunction with bioaffinity sensors is molecular imprinting, which has been used for inducing affinity sites towards specific compounds in synthetic materials. The process entails polymerisation around a (template) molecule, the so-called ‘print species’, using monomers with chemical functionalities that are complementary to those of the print species. Subsequent removal of the template exposes recognition sites within the polymer, which possesses ‘memory’ of this molecule in terms of shape and electronic structure [7].

**Enzyme biosensors**

Glucose sensors based on glucose oxidase in an electrochemical sensor (Figure 3b) continue to comprise the largest percentage of biosensors on the market because of their use in the diagnosis of diabetes. Continuous effort is being expended on implantable sensors. Except for the long-term transduction of biopotentials from myocardium and skeletal muscle, there are, as yet, no long-term implantable sensors available that are of use clinically. New developments in long-term glucose sensor implants are still based on the enzyme electrode principle but these implants have shown instances of remarkably stable performance for three months or more [8].

Improvements of in vitro sensors for glucose and other analytes such as lactose (which is interesting in body fluids [e.g. muscle control while training] and in food) are mainly based on new immobilisation techniques that allow for a direct electron transfer to the electrode. In particular, Willner and co-workers [9] introduced new interface chemistry concepts in which apoenzymes are reconstituted onto an electrode surface modified with their corresponding cofactor.

**Transmembrane sensors**

The basic practical problem in preparing transmembrane devices is the spreading of a biological membrane without destroying its activities; achievement of a sufficient electrical insulation of the planar membrane at the transducer is also a problem. Therefore, black lipid membranes (BLMs, dense lipid bilayer membranes that appear black) or tethered membranes based on phospholipids are used as the matrix in which natural receptors are reconstituted. This approach still lacks considerable progress because functional reconstitution, in particular of membrane-spanning proteins such as ion channels, cannot yet be established for stable immobilised membranes. The main problem concerns achieving a sufficient lack of defects (which minimises leakage of molecules through the membrane), adequate reservoir capacity for ions/molecules between membrane and transducer and long-term stability.
During the past year successful examples for transmembrane sensors were shown, however, in particular by Cornell and co-workers [10,11]. A synthetic ligand-gated ion channel switch was co-assembled with a tethered BLM, based on novel sulfur-containing lipids. On the basis of the bacterial ion channel gramicidin, the switch operates by preventing the alignment of monomeric gramicidin into membrane-spanning dimers, which are conductive. Channel switching may be achieved with most types of receptors, including antibodies and nucleotides.

A new approach to tethered membranes was used in the functional immobilisation of the nicotinic acetylcholine receptor (nAChR), where membranes were supported on gold surfaces using so-called ‘thiolipids’, which are specially synthesised phospholipids bearing in the headgroup a thiol group attached to the terminus of a long polyethyleneoxide spacer. Surface plasmon resonance spectroscopy was used as a sensing technique [12]. (Rapid synaptic signal transmission between cholinergic muscle and nerve cells is mediated by the nAChR. Sensors that measure changes in the receptor after application of certain analytes give new insights into receptor function.)

**Cell sensors**

Perfect spreading of biological membranes onto thin film substrates is still extremely difficult to achieve; more sophisticated biosensors, based on the use of whole cells that are specialised towards certain metabolic functions, have been developed as an alternative. Of particular interest are experimental setups by which signals are deduced without destruction of the cell. This may be realised either electrically or optically.

The most commonly used optical method is to monitor intracellular species with fluorescent labels. In a new approach, fibreoptic as well as cell-implantable nanosensors have been developed and tested for pH, calcium, sodium, potassium, nitric oxide, oxygen and glucose detection for their use in early rat embryos and single mammalian cells. The problems of fragility, photobleaching, leaching and invasiveness have been largely overcome. The selectivity and signal : noise ratio are adequate for intracellular work. These real-time millisecond response, supersensitive chemical sensors are aimed at the refinement and acceleration of biomedical research [13].

Optical imaging fibers may be implemented in the direct chemical analysis of single cells. These fibres comprise thousands of individual fibers that have been melted and drawn together in a coherent manner such that each fiber in the bundle carries its own isolated optical signal from one end of the fiber to the other. Through a wet-etch process that takes advantage of the differences in etch rate (the layer thickness that is removed in a certain time, which depends on the material) between core and cladding materials, individual femtolitre-sized wells are formed at the distal tip of an optical imaging fibre. The wells are fabricated so that the well diameter and depth allow for the accommodation of one cell per well. Fluorescently-labeled NIH 3T3 mouse fibroblast cells are dispersed into the well array by allowing a suspension of cells held above the array to settle into the wells and adhere to the well bottom. The pattern of the cells populating the wells is determined by exciting the fluorescent cell membrane label at the appropriate wavelength. Once the location of the cells in the array has been determined, fluorescence measurements of an analyte may then be made at other wavelengths. The chemical environment of each cell is directly monitored by correlating the release or consumption of a specific analyte with a change in fluorescent intensity [14].

In another approach cellular functions and, in particular, cell--cell attachment are measured by interdigitated electrodes and, depending on the application, other different types of sensors (i.e. ion-sensitive field effect sensors, oxygen sensors or temperature sensors) [15].

More sophisticated setups aim at understanding the responses of individual cells and at understanding cell--cell communication, for instance in neural networks. Biological neuronal networks are very sensitive to changes in their chemical environment. The response of the neuronal network is often substance-specific and concentration-specific. Employing appropriate data processing and analysis, these biological systems could be used (for certain sensory tasks) as network biosensors. For example, electrophysiological network activity patterns of spinal cord cell cultures were recorded via an array of 64 photoetched electrodes [16]. Changes in the spontaneous activity because of the addition of different concentrations of strychnine could be evaluated quantitatively by artificial neural network analysis ([17,18]. Because a huge number of molecules influence in a specific way the electrical activities of cells that have certain receptors, this approach offers great flexibility for future work.

Even whole animals or certain body functions of animals are used in current biosensors. Recent results indicate the possibility of using insect olfactory organs as the sensing part of a novel type of biologically sensitive field-effect transistors. One promising goal of this approach is the design of biosensors utilising the considerable potential of the highly optimised chemoreceptors seen in more than one million insect species [19]. Several applications have been shown so far, including detection of plant damage in glasshouses [20] and detection of smouldering coal [21].

**Conclusions and outlook**

In recent years, biochemical sensors have been shown to provide complementary and additional information to that contributed by the well-established bioanalytical techniques. Particular advantages of biochemical sensors concern the following: the possibility of miniaturising the
setup, in principle down to the molecular scale; the use of well-established microsystem technologies during manufacture (of at least certain sensor components); integration of signal preprocessing steps on a chip; and the building of arrays for more complex pattern recognition analysis.

Major progress has been achieved by improvements in our molecular understanding of surface modifications, with particular emphasis on artificial biosensing interfaces. These artificial biosensing interfaces link biosensor-active coatings (containing either synthetically manufactured ‘biomimetic’ or natural biological function units) with inorganic transducer substrates. One new trend concerns the patterned immobilisation of biomolecules on surfaces. Microcontact printing of proteins has proven to be an excellent means for direct patterning of biomolecules on solid substrates. In this technique, a hydrophobic elastomeric stamp is made from poly(dimethylsiloxane), which bears a structural surface. Monolayer quantities of protein can be immobilised on this surface. These biomolecules can transfer with >99% efficiency from the stamp to a substrate after just ~1 s of contact. This printing process allows the creation of conventional (fluorescence and enzyme-linked), as well as novel, assay formats at scales that involve the placement of <1000 molecules in well-defined locations on a surface [22].

Such formats may be used in bioanalytical microsystems that comprise another new trend in bioanalysis. In such microfluidic systems, miniaturised biosensor arrays, as well as miniaturised sampling, filtering, and so on, has to be accomplished. Although large problems still exist (e.g. concerning reliable long-term stable pumps), first examples of successful applications are emerging [23,24].

A variety of transducer principles have been, and new ones will be, tested for their use in biosensing. Further progress is expected from merging the classical sensor technologies with new technologies in scanning probe techniques. The tip of an atomic force microscope (AFM), for instance, may be used to monitor forces between complementary biomolecules [25]. These experiments were carried out to characterise antibody–antigen binding and made it possible to detect the presence of individual antigen molecules. AFM can act as an extraordinarily sensitive biosensor because it can detect single antibody–antigen bonds, and by measuring their strength, distinguish them from nonspecific background forces or other forms of interference. Two new immunosensors are under development that use this ‘force discrimination’ principle, but without the extensive instrumentation of an AFM, to achieve sensitivity approaching detection of a single molecule. These sensors, the force amplified biological sensor and the bead array counter, use antibody–antigen bonds to immobilise magnetic microparticles on a solid surface. When a magnetic field is applied, the particles break the specific and/or nonspecific bonds. Force, magnetic field or optical transducers determine the number of particles that remain on the surface under these conditions [26].

In view of microelectronic compatibility, electrical signal transduction principles should be favoured in long-term developments. In many cases, however, the corresponding interface stabilities cannot, as yet, be achieved satisfactorily. In this situation, optical transducers have become extremely important, although in the long-term electrical transducers involving not only electrons, but also ions, will become of great importance. The involvement of ions is a prerequisite for many new applications. One example concerns the often desired direct link between mammad computers (based on hardware) and nerve systems (based on wetware i.e. the use of biological materials in a wet environment, as opposed to a hard [silicon] environment). Bridging this interface requires the building of reliable hybrid systems. This requires a fundamental understanding of bioelectronics principles, including signal generation, transfer, recording and storage [27,28].

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


