TECHVIEW: BIOCHEMISTRY

Biosensors—Sense and Sensitivity

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Although the charge-couple device (CCD) chip such as that used in video cameras arguably provides an artificial eye on our world, we seem to be missing effective electronic versions of our other senses. Whether the research area is drug discovery, proteomics, or environmental monitoring, there is a need for escalating quantities of molecular information to feed hypotheses and to support or abandon models. Biosensors (*1*)—which fuse the exquisite sensitivity and specificity of living systems with the processing power of microelectronics—are simple, inexpensive measurement systems that may be able to provide this information.

The earliest biosensors were catalytic systems that integrated enzymes, cellular organelles, tissues or whole microorganisms with transducers that convert a biological response into a digital electronic signal. The principal transducers used were electrochemical, optical, or thermometric. The next generation of biosensors, affinity biosensors, capitalized on a similar range of measurement principles but with the addition of piezoelectric transducers (that interconvert mechanical deformation and voltage to measure mass or viscoelastic effects) and magnetic transducers. Affinity biosensors deliver real-time information about the binding of antibodies to antigens, cell receptors to their ligands, and DNA and RNA to nucleic acid with a complementary sequence. There are multifarious applications of both types of sensor—for example, they can be used to measure blood glucose levels, detect pollutants and pesticides in the environment, monitor food-borne pathogens in the food supply, or to detect biological warfare agents. The future promises high-density arrays of biomolecular sensors that rival microelectronics in size and capacity, deliver the recognition and discrimination of complex analytical equipment, and furnish scientists with biologically relevant information.

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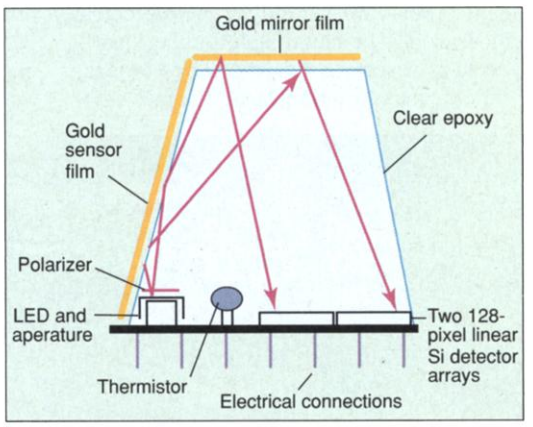
Enzyme Electrodes

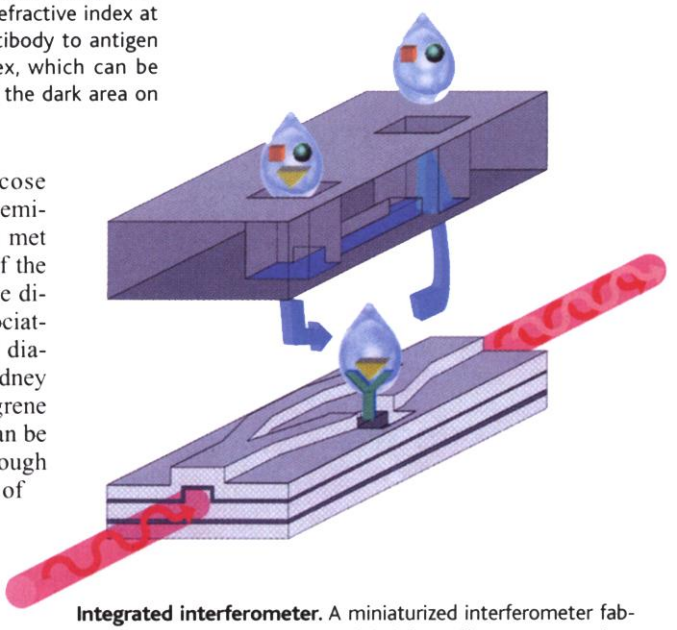
Historically, glucose sensing has dominated the biosensor literature and has delivered huge commercial successes to the field. The deceptively simple combination of a fungal enzyme (glucose oxidase) with an electrochemical detector has effectively met the needs of the 1 to 2% of the world's population that have diabetes. Complications associated with insulin-dependent diabetes such as blindness, kidney and heart failure, and gangrene (resulting in amputation) can be reduced by up to 60% through stringent personal control of blood glucose, including frequent monitoring of glucose levels. People with non–insulin-dependent (Type II) diabetes can also benefit from strict monitoring of glucose levels. Enzyme-based electrode biosensors have been used to test glucose levels in blood samples since 1975, but the emergence of a convenient, hand-held commercial format in 1987 revolutionized their use (*2*). The original hand-held glucose biosensor was a pen-shaped instrument with disposable electrodes; subsequent versions resembled credit cards, a computer mouse, and the popular electronic pet, the Tamagotchi. All of these hand-held glucose biosensors offer ergonomic elegance and a convenient small size while keeping the blood sample away from the readout device and, hence, preventing contamination of the instrument.

The four companies now dominating the world market in hand-held glucose biosensors have adopted the principle of mediated amperometric enzyme electrodes. In these catalytic biosensors, oxidation of the analyte (in this case glucose in a blood sample) is catalyzed by an oxidoreductase enzyme (normally glucose oxidase) and the electrons liberated are shuttled to the electrode through artificial electron acceptors or mediators such as ferrocene, osmium derivatives, or hexacyanoferrate. This mediation produces a current that is directly proportional to the concentration of the analyte.

Although the development of more convenient hand-held glucose biosensors for one-shot measurements of glucose in a pin prick of blood have been of enormous help to diabetic patients, it is clear that further improvements in the technology are essential. There are two principal avenues of de-



The sprightly *Spreeta*, a chip-based SPR sensor. Light generated by the LED and polarizer is totally internally reflected, creating surface plasmon resonance in the gold sensor film. The angle at which maximum light energy is absorbed is dependent on the refractive index at the sensing surface. The binding of antibody to antigen results in a change in refractive index, which can be measured by recording the position of the dark area on the detector array.



Integrated interferometer. A miniaturized interferometer fabricated in silicon operates as a label-free affinity sensor. When the immobilized antibody on the surface of the device binds its complementary antigen in the sample, a change in refractive index occurs. This change affects the propagation of light in the sensing arm resulting in interference when the beams combine.

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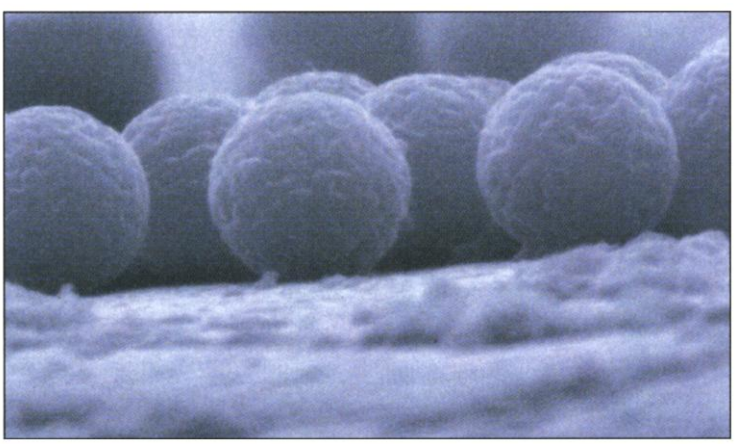
velopment: implantable subcutaneous glucose electrodes, and minimally invasive or noninvasive instruments for glucose measurement. Both approaches offer more detailed information about the rapid fluctuations in glucose levels in the body and have recently obtained approval from the U.S. Food and Drug Administration (FDA) for use in patients. Microfabrication technology has aided in the design of enzyme electrodes that can be inserted under the skin, typically in the abdominal area (*3*). A monitor attached to the patient receives a measurement from the biosensor every ten seconds and stores an average glucose value in its memory once every five minutes. This implantable sensor has a lifetime of up to three days. An alternative approach uses reverse iontophoresis to extract glucose from skin tissue and to measure amperometrically the hydrogen peroxide resulting from oxidation of the glucose in the presence of glucose oxidase (*4*). In this case, the instrument is in the form of a wrist watch providing automatic readings up to three times per hour for as long as twelve hours. The success of enzyme electrode biosensors for detecting glucose levels has prompted the introduction of similar devices to measure lactate, creatinine, or urea in the blood or urine of critical-care patients.

Mass fabrication methods have enabled biosensors to be produced in large volumes for clinical markets. Two techniques borrowed from the electronics industry have proved particularly important—screen printing (ink is pressed through a mask to form a pattern on a ceramic or plastic base) and photolithography (a photoresistant material is exposed to ultraviolet light passed through a mask and then the silicon is chemically etched). Reproducible manufacture of biosensors with screen printing techniques means that each instrument does not have to be calibrated before use.



A magneto-immunosensor. Electron micrograph of paramagnetic beads showing that an affinity reaction has occurred. To monitor antigen-antibody interactions, one partner can be attached to the bead and the other to a solid surface.

Affinity Biosensors

Whereas the preferred method of measurement for catalytic biosensors is electrochemical, affinity biosensors have generally proved more amenable to optical detection methods. The commercialization of real-time bioaffinity monitors based on surface plasmon resonance (SPR) has provided a powerful new tool to the research community and to the pharmaceutical industry in particular (*5*). Commercial SPR devices detect alterations in the optical evanescent waves that result from small changes in refractive index at the interface between the sample and the device, which are caused by, for example, antibody binding to antigen. Recent advances in optical affinity biosensors include the packaging of SPR in a small, inexpensive chip (Texas Instruments' *Spreeta*) (see figure, top, previous page) and the development of integrated Mach Zehnder interferometers fabricated in silicon (see figure, bottom, previous page). The advantages of these label-free biosensors—which detect a binding event directly by monitoring the change in a physical property such as refractive index rather than indirectly with a fluorescent label—are shared by piezoelectric biosensors. The latter exploit either quartz crystals or surface acoustic waves to measure the mass or viscoelastic changes associated with affinity reactions between one molecule and another.

Optical evanescent-wave technology has also been used to streamline the design of affinity biosensors that contain a label or marker. For example, many immunoassays use a fluorescent marker to indicate when antibody binds antigen. An impressive recent example is provided by immunosensors designed to detect microbial warfare agents (*6*). The integration of photochromic dyes into the antibodies of immunoassays has facilitated the production of high-affinity sensors that can be optically switched to low affinity so that the devices can be regenerated and used again (*7*). A recent advance in affinity biosensor immunoassays is the introduction of paramagnetic particles attached to antibodies as the label. Binding of antibody to antigen attached to a solid substrate, can be detected with an electronic device that measures the magnetic field induced by the paramagnetic beads (see the figure below). The detector can be fabricated into a small hand-held device, and the approach offers the added bonus of providing a permanent record because the contents of the assay stick can be remeasured at any time, like a piece of magnetic recording tape.

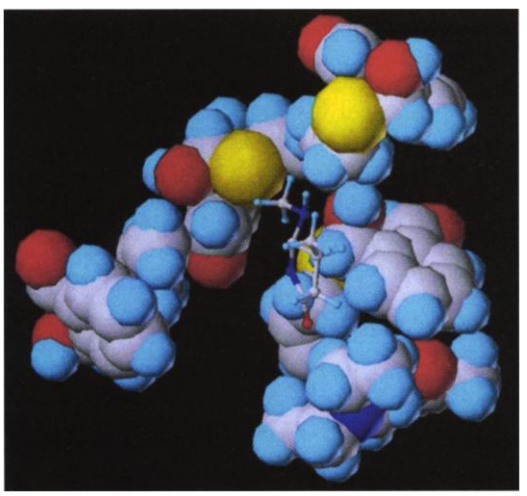
Affinity biosensors with electrochemical measurement devices offer some of the most sensitive immunoassays currently available. By exploiting enzyme amplification in low-volume capillaries, they can measure as little as 10^{-21} moles of analyte (*8*). A very recent award-winning innovation comes from the laboratories of Schumann and Csoregi (*9*), who have developed an affinity biosensor in which the diffusion coefficient of an electroactive label alters upon recognition of its complementary binding partner in an antibody-antigen reaction. The increase in the molecular weight and the concomitant decrease in the diffusion coefficient of the label are monitored by determining the diffusion-limited current by cyclic voltammetry. Signal amplification is obtained by redox recycling at a microelectrode. Another elegant approach has been to develop ion-channel biosensors that can measure picomolar quantities of proteins (*10*). In these microfabricated devices, the conductance of a population of molecular ion channels is switched by molecular recognition events such as antibody binding to antigen or DNA binding to its complementary nucleic acid.

The rapid evolution of nucleic acid–based assays in the form of DNA chips is one of the latest developments in biosensor research. The concept of a million hybridization assays performed simultaneously on a one-square centimeter planar chip has much in common with the goal of high-density sensor arrays. Although the transducer in many DNA chips is not yet fully integrated, the commonality of the technologies is apparent, and a number of experimental nucleic acid biosensors have been described in the literature linked to electrochemical, optical, or piezoelectric detection methods (*11*).

Monitoring the Environment

Although commercial pressures have driven the demand for biosensors in the medical and pharmaceutical sectors, public concern for the environment has stimulated the application of biosensors to the measurement of pollutants and other envi-

ronmental hazards. Chemical warfare agents and, more recently, pesticides have been detected by biosensors in which the offending substance inhibits enzymes such as acetylcholine esterase. Electrochemical detection of the products of this enzyme reaction have been combined with the screen-printing technology developed for the glucose sensor to provide one-shot, portable biosensors for environmental monitoring of pesticides. Pioneering work by Karube's group in Japan in the late 1970s resulted in the development of biochemical oxygen demand (BOD) whole-organism environmental biosensors. These BOD sensors offer a rapid twenty-minute method to determine the amount of metabolizable organic material in waste water by measuring oxygen consumption by immobilized bacteria or yeast. Biosensors in which bacteria (genetically modified to express the *lux* gene) glow in response to pollutants have provided important information about these chemicals. A replacement for the Ames test for detecting genotoxic molecules has been constructed in yeast using a green fluorescent protein reporter gene (*12*) coupled to a chip-based flow cytometer. Single yeast cells that have had their DNA repair mechanisms switched on can be detected by virtue of their fluorescence in this miniaturized instrument. DNA technology has also been exploited for engineering recombinant antibody fragments that are robust enough but still sufficiently sensitive to be components of biosensors for detecting low molecular weight pollutants, such as triazines, at concentrations in the parts per trillion range (*13*).



Bite-and-switch. A bite-and-switch receptor designed by molecular imprinting for detecting creatinine and creatine in clinical blood samples. The analyte and the receptor are shown by the stick-and-ball structures, respectively. The primary amine of the analyte reacts with the receptor to form fluorescent isoindole.

Synthetic Receptors

The design of semi-synthetic receptors in biosensors may be superseded by totally synthetic ligands produced with the aid of computational chemistry, combinatorial chemistry, molecular imprinting, self assembly, rational design, or combinations of one or more of these. For example, molecularly imprinted polymers (MIPs) have attracted a lot of attention because they can behave as artificial receptors for molecular recognition. The striking resemblance of the binding properties (affinity and specificity) of MIPs to those of natural receptors, together with their inherent stability, low cost, and ease of preparation for industrial application have made them an attractive alternative to antibody-based technologies. Conventional imprinted polymers are usually synthesized empirically or semi-empirically using a limited number of common monomers in a trial-and-error approach. At Cranfield, we have used computer modeling to study the interaction between target analytes and a virtual library of monomers, thus guiding the selection of monomers for the preparation of MIPs with higher selectivity and affinity for the analyte. A further challenge is to transduce the binding event into a detectable signal. This has been achieved with both optical and electrochemical configurations. In one example, a "bite-and-switch" approach has been used to produce sensors that detect creatine and creatinine in blood (see the figure above). In this two-step recognition process a broadly specific chemical reaction is complemented by a three-dimensional recognition pocket to produce a strong "bite," which is followed by a "switch" to the fluorescent form of the indicator. A thioacetale reaction—between the polymer and the amine groups in creatine and creatinine—results in the formation of a fluorescent isoindole complex; this reaction was made more specific for creatine and creatinine by molecular imprinting (*14*). In a further example of a screen-printed design, an electrochemical sensor was developed that detected the herbicide 2,4-D by the displacement of homogentisic acid from a MIP (*15*).

Conclusions

Biosensors for monitoring blood glucose at home have achieved prominence in the world diagnostics market and are now being joined by a diverse array of biosensors for detecting other analytes of clinical importance. However, the commercial success of other types of biosensor has been hampered by high development costs in relation to market size and the lack of suitable biological recognition molecules that are inexpensive to produce and stable enough to withstand storage. This problem becomes particularly acute when designing high-density analytical arrays to support future needs in medical diagnostics, functional genomics, proteomics, drug discovery, environmental monitoring, food safety, process control, and defense. DNA technology has furnished one powerful way to increase natural diversity and create organisms with new metabolic activity or libraries of new receptors for integration into sensors. Biomimetic sensors (those containing synthetic receptors that mimic the recognition properties of biological molecules) may provide a viable alternative to solving these problems. Whichever type of receptor becomes dominant, the volume of data from sensing "chips" is set to increase dramatically. We will need to move rapidly from the concept of single numerical values for expressing our measurements to the adoption of pattern recognition and information extraction, if we are to make effective use of the data that biosensor technology should soon make available to us. We have had a microprocessor revolution and we are about to see this complemented by a microanalytical revolution. The analytical power unleashed will have enormous impact on our daily lives by altering our ability to monitor and predict susceptibility to and effects of disease and the environment, hence leading to an improved quality of life for all.

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