

Biosensors: Introduction

Overview

A biosensor is a device that - in some way - makes use of a biological detection system. Biosensing is the process of using biosensors to gather information about living systems.

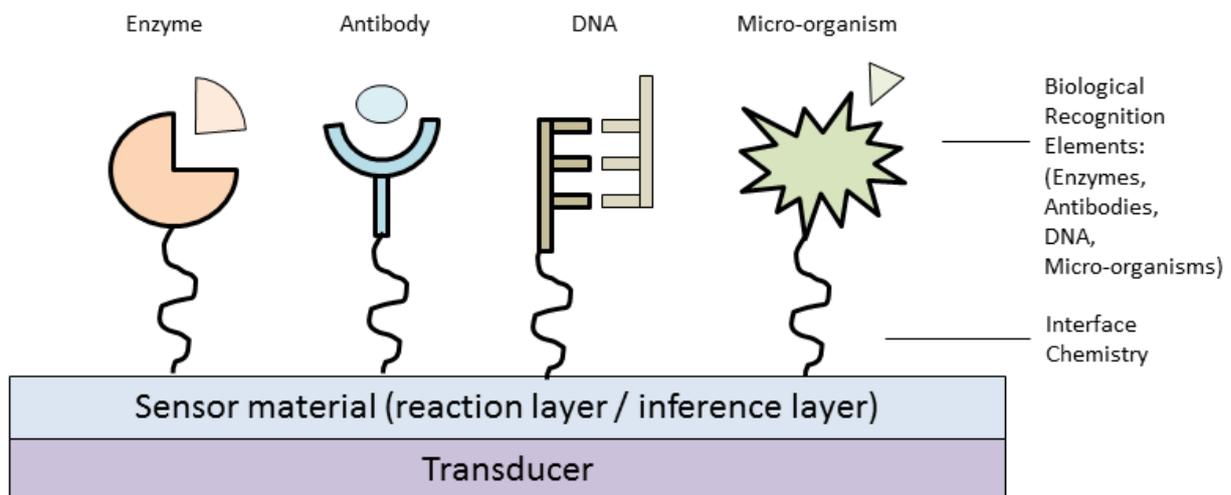
The precise interpretation of the term 'biosensing' depends on the research domain. In embodied robotics, for example, biosensing is interpreted as the various ways in which natural systems 'sense' their environments. Understanding these processes is important to this domain as embodied robotics seeks to learn from life-like systems. In other engineering domains biosensing can have a different meaning. It can be understood as a sensing process that incorporates biological material or reactions into a sensor, either to improve an existing system, add new features, or create a completely new sensor - even one that might not exist as such in existing living systems.

While silicon-based electronics is governed by principles of materials science, biosensor transducers are additionally subject to biological principles. A biosensor can include, for example, an enzyme, an antibody, or a microorganism that in turn is connected to an electronic element.

With this in mind one could define a biosensor as a self-contained integrated device consisting of a biological recognition element (enzyme, antibody, DNA or microorganism) which is interfaced to an analytical device and together respond in a concentration-dependent manner to a given analyte.

Thévenot's definition of a chemical biosensor from 2001 is often quoted in biosensor research: "An electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element." (The first biosensor was developed in 1962 by Clark and Lyon as an 'enzyme electrode' for the measurement of glucose levels.)

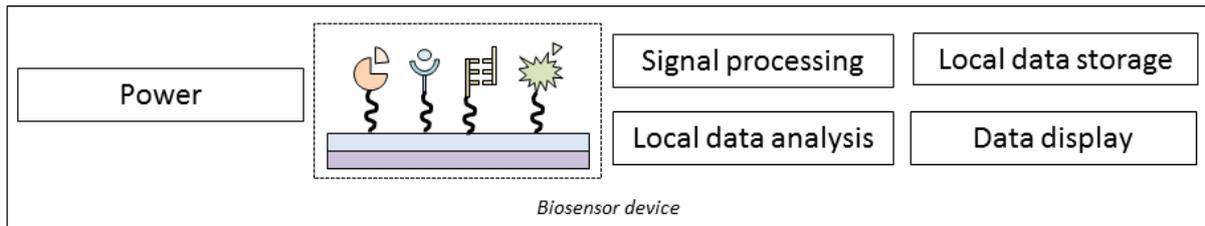
The diagram below shows the major elements of a biosensor:



While biosensors constitute a fairly new sensor class, the basic principles by which biosensors operate are the same ones that govern well-known electronic sensors. They are also subject to the same performance criteria as electric sensors (sensitivity, selectivity, accuracy, precision, repeatability, noise tolerance etc.).

Like other sensors, biosensors ‘transduce’ or translate one kind of energy into another and must be placed into a chain of components that detect, translate and modify an original ‘sign of life’ into a useful signal. For example, a cadmium sulfide sensor changes its electric resistance as a function of the (visible and near infrared) light energy impinging on its surface due to the material physics of thin film cadmium sulfide. Placing such a light sensitive element into a battery powered circuit alters the flow of electricity, and this can be used to infer (through an appropriate algorithm) the current local light conditions, and (with some data analysis) the change of light conditions over time.

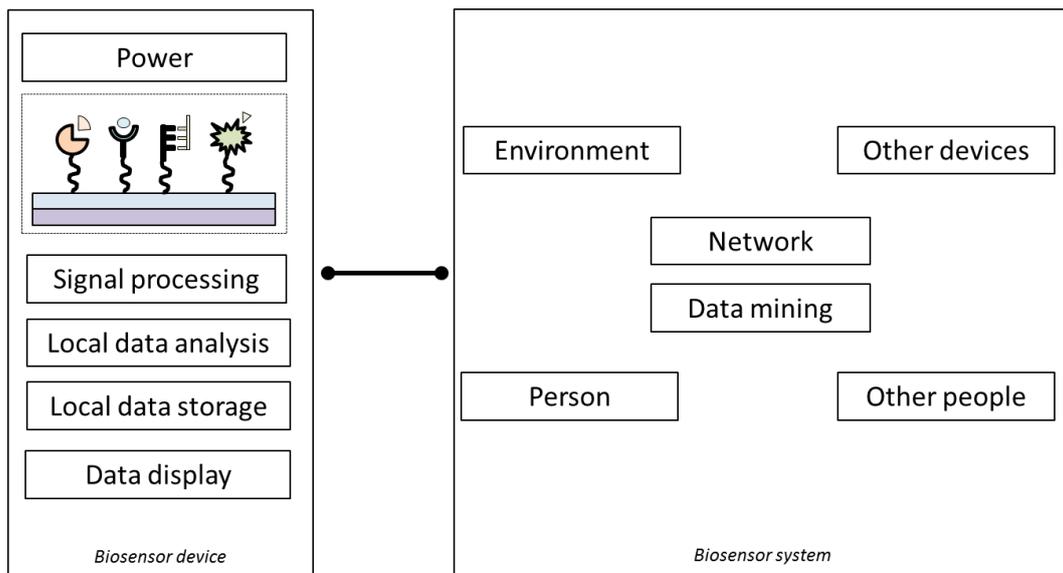
The diagram below shows a biosensor and its enabling components:



Biosensors are usually classified into various groups either by type of transducer employed (electrochemical, optical, piezoelectric, and thermal) or by the kind of bio-recognition element utilized (antibody, enzymes, nucleic acids, and whole cells). Both components of the biosensor, namely, bio recognition element (referred as a receptor) and transduction platform (referred as a transducer) play an important role in the construction of a sensitive and specific device for the analyte of interest (referred as a target).

In SiReBi we will be focusing on biosensors and biosensing as sensor systems that ‘make use of biological principles’ and ‘sense biological processes’ to gather information about life forms, and in particular people.

Biosensors are important today because they can sense what many other traditional sensing systems cannot. Many biosensors can be industrially produced (in scale and cost). Also, they can be networked to produce large scale multi-valued sensing systems.



Biosensor categories

Biosensor operations are dependent on the details of the sensor design, just as chemical sensors are. A chemical sensor is a device that transforms chemical information into an electrical signal. This principle is based on the fact that a constant potential applied between a working and a reference electrode promotes a redox reaction, which produces a current. The magnitude of this current is proportional to the concentration of electro-active species present.

Biosensors use a biochemical recognition mechanism, such as an analyte concentration, in direct contact with a transducer. In all cases the binding of an analyte changes the physical-chemical properties of the sensor. The transducer, analyte and the binding mechanism are chosen such that the changes are measurable. For example, the measurement of small changes in mass is a transduction form that has been used for biosensor development. More generally, a biosensor recognizes and converts a biological event into a detectable signal by the action of a receptor, transducer and a processor.

Enzymatic biosensors measure the selective inhibition or the catalysis of enzymes by a specific target. Whole-cell biosensors detect responses of cells after exposure to a sample, which are related to its toxicity. Affinity-based recognition elements bind to individual targets or groups of structurally related targets. Affinity-based sensors are very sensitive, selective and versatile since affinity-based recognition elements can be generated for a wide range of targets

Optical transducers, for example, are based on various technologies of optical phenomena, which are the result of an interaction of an analyte with the receptor part. Reflectometric techniques are based on a white incident light passing the interface between different refractive indices, which will be reflected in part. These reflected beams superimpose and build a characteristic interference spectrum. The binding of a biological receptor such as an antibody to the surface changes the thickness of the layer through which light passes and causes a change in the reflectance spectrum.

Biosensor signal detection methods

Below is a short list of some of the detection methods (with advantages and disadvantages) used in biosensors:

Amperometry is operated at a given applied potential between the working electrode and the reference electrode, and the generated signal is correlated with the concentration of target compounds. In the amperometric detection, the current signal is generated as a function of the reduction or oxidation of an electro-active product on the surface of a working electrode.

Conductometry is a technique depending on the conductivity change in the solution due to the production or consumption of ions, for example, by the metabolic activity of microorganisms. The measurement of conductance can be fast and sensitive, making conductometric microbial biosensors very attractive. Such biosensors are suitable for miniaturization since they require no reference electrode in the system. However, the selectivity of conductometric biosensors is relatively poor.

Potentiometry involves the measurement of the potential difference between the working electrode and the reference electrode which is dependent on concentration-related behavior. The transducer employed in the potentiometric technique is usually a gas-sensing electrode or an ion-selective electrode. The sensitivity and selectivity of potentiometric biosensor are outstanding due to the species-selective working electrode used in the system. However, a stable and accurate reference electrode is required.

Voltammetry is the most versatile technique in electrochemical analysis. Both the current and the potential are measured. The position of peak current is related to the specific chemical and the peak

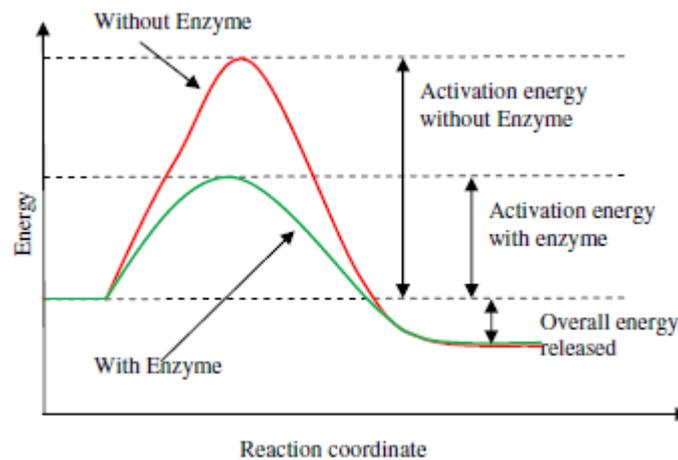
current density is proportional to the concentration of the corresponding species. A remarkable advantage of voltammetry is the low noise which can endow the biosensor with higher sensitivity. In addition, voltammetry is able to detect multiple compounds, which have different peak potentials, in a single electrochemical experiment (or scan), thus offering the simultaneous detection of multiple analytes. The voltammetric technique is one of the most sensitive electro-analytical methods.

Optical detection is usually based on the measurement of luminescent, fluorescent, colorimetric, or other optical signals produced by the interaction of microorganisms with the analytes and correlates the observed optical signal with the concentration of target compounds. Genetically engineered microorganisms have been widely applied in optical whole-cell biosensors. A reporter gene is fused with an inducible gene. In the presence of a target analyte, the inducible gene is activated and then activates (“turns on”) or represses (“turns off”) the expression of a reporter gene which is responsible for the production of a measurable optical signal. Optical sensing techniques are especially attractive in high throughput screening since they enable biosensors to monitor multiple analytes simultaneously.

Biosensors: Enzymes and DNA

Enzymes

Enzymes are folded polypeptides (polymers of amino acids) which catalyze chemical reactions without being used up in the conversion of substrates to products. During this process an enzyme temporarily binds up with the substrate molecules and makes an intermediate compound thereby lowering the activation energy required for the total reaction:



Enzymes are commonly used to generate electrical signals for detection of DNA hybridization (the degree of similarity between pools of DNA). The enzyme, previously bound to the DNA probe, triggers the catalysis of a redox reaction and further generates an electrochemical change due to the hybridization event

When an enzyme is immobilized onto the substrate, the enzyme molecules get attached to the substrate (they are then called “active sites”). If the number of active sites occupied by the substrate molecules is low (low concentration), first order kinetics prevail, and substrate concentration is directly proportional to the reaction rate. At high substrate concentrations, all of the active sites of enzyme molecules are filled and the reaction rate is independent on substrate concentration. A further increase of substrate concentration then leads to saturation in the reaction rate.

Major advantages of enzymes include the fact that they are the most widely used recognition element due to their unique specificity and sensitivity. However, they have several disadvantages: Purification of enzymes is costly and time-consuming. In addition, the *in vitro* operating environment can result in

reduced enzyme activity. Also, there are a limited number of substrates for which enzymes have been evolved, and there are to date few tested interactions between environmental pollutants and specific enzymes.

DNA

Most DNA sensor platforms are based on the hybridization concept. DNA hybridization is usually issued between a known DNA sequence (probe) and an unknown counterpart (target). The biomolecular recognizing event occurs directly on the surface of a physical-transducer, and the immobilized DNA-chain is a part of the biosensor itself. Nucleic acid hybridization is stronger and more specific when the complementarity degree between two DNA chains increases. The specificity and stability of the linkage reach a maximum in the case of full (100%) complementarity (= 'recognition'). Unlike enzymes and antibodies, nucleic acids from biological recognition layers are easily synthesizable in the laboratory and stable.

In electrochemical DNA biosensors, a single-chain of DNA is immobilized onto an electrically active surface (electrode). Measurable changes in electrical parameters (e.g., current, potential, conductance, impedance and capacitance) are caused by the hybridization reaction in the biological layer.

Examples

An enzyme-based glucose biosensor

An example of an enzyme-based biosensor is the glucose biosensor for diabetes management which utilizes the enzyme glucose oxidase. This enzyme oxidizes blood glucose to glucono-lactone and hydrogen peroxide to produce a measurable signal. Glucose biosensors are mostly electrochemical in nature as the mechanism involves oxidation and reduction of the sample and the enzyme. Enzyme-based biosensors are highly selective, fairly fast-acting and are thus suitable for applications such as diabetes care. However, they suffer from loss of activity over time due to denaturation.

A DNA-based piezoelectric biosensor

A DNA sequence with a few hundred base pairs usually possesses a sufficiently high molecular weight so that the mass increase caused by hybridization of a DNA-chain with its complimentary counterpart previously immobilized on the surface of a piezoelectric quartz crystal may be specifically correlated with the increase in the fundamental resonance frequency of the crystal.

Glossary

Sources:

- 1) <http://bayley.chem.ox.ac.uk/teaching/> (referencing "Biochemistry" 5th edition 2002, JM Berg, JL Tymoczko, L Stryer Freeman)
- 2) <http://www.chemguide.co.uk/> and
- 3) Wikipedia

Amino acid

Building blocks of all proteins. Amino acids contain an amino group (NH₂), a carboxylic acid group (COOH), and are linked together form proteins.

Analyte

A substance of interest, to be measured or evaluated.

Antibody

A large Y-shaped protein used by the immune system to identify and neutralize foreign objects such as bacteria and viruses.

Antigen

A substance or molecule that, when introduced into the body, triggers the production of an antibody

Denaturation

A process in which proteins have their structure compromised by external influences (heat, solvents, salts, etc.). Denatured proteins can exhibit a wide range of characteristics, from loss of solubility to communal aggregation (as in a cooked egg). In living cells, denaturation can lead to cell death.

DNA

A nucleic acid made of sugar (deoxyribose), phosphate and nucleobase, with a sugar-phosphate backbone and 4 different bases: Adenine, Thymine, Guanine, Cytosine. They encode protein sequences and genes. Double stranded helix with paired bases.

Enzyme

A protein that catalyzes (increases the rate or reduces the reaction energy required) chemical reactions

Hybridization

The binding of two complementary strands of nucleic acids to a single element, called a hybrid

Lipids

A wide collection of molecular bilayers (fats, waxes, some vitamins) that encapsule cells.

Nucleic acid

Protein sequences such as DNA, RNA

Oxidation

One side of a chemical reaction (redox) that increases oxidation (increases oxygen content) through the loss of electrons. Example: oxidation of carbon creates carbon-dioxide.

Piezoelectric sensor

A piezoelectric sensor employs materials that resonate under the application of an external alternating electrical field. Typically, quartz crystals are utilized, producing an oscillating electric field in which the resonant frequency of the crystal depends on its size, shape and mass.

Proteins

Folded 3D structures built from 20 base amino acids. They operate as structural components, binding molecules, and catalysts.

Reduction

One side of a chemical reaction (redox) that decreases oxidation (reduces oxygen content) through the gain of electrons.

Ribosome

An internal component of a cell that assembles the 20 base amino acid molecules to form the particular protein molecule in response to the nucleotide sequence of RNA.

RNA

Nucleic acid similar to DNA, but single stranded, with a different sugar (ribose instead of deoxyribose), and a different nucleobase (uracil instead of thymine). RNA is responsible for transferring genetic information from DNA to the cell ribosomes.

This introduction is based on information from the following sources (available in SiReBi):

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