Biosensors and Biodetection

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METHODS IN MOLECULAR BIOLOGY™

Biosensors and Biodetection

Methods and Protocols
Volume 504: Electrochemical and Mechanical Detectors, Lateral Flow and Ligands for Biosensors

Edited by

Avraham Rasooly* and Keith E. Herold†

**FDA Center for Devices and Radiological Health, Silver Spring, MD, USA*
and
National Cancer Institute, Bethesda, MD, USA
† Fischell Department of Bioengineering, University of Maryland, College Park, MD, USA

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Editors
Avraham Rasooly
FDA Center for Devices
and Radiological Health
Silver Spring, MD
USA
and
National Cancer Institute
Bethesda, MD
USA
rasoolya@mail.nih.gov

Keith E. Herold
Fischell Department of Bioengineering
University of Maryland
College Park, MD
USA
herold@umd.edu

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Preface

1. Biosensor Technologies

In recent years, many types of biosensors have been developed and used in a wide variety of analytical settings, including biomedical, environmental, research, and others. A biosensor is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a "device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles, or whole cells to detect chemical compounds usually by electrical, thermal, or optical signals" *(1)*. Thus, almost all biosensors are based on a two-component system: a biological recognition element (ligand) that facilitates specific binding to or biochemical reaction with a target, and a signal conversion unit (transducer). Although it is impossible to fully cover the fast-moving field of biosensing in one publication, this publication presents some of the many types of biosensors to give the reader a sense of the enormous potential for these devices.

An early reference to the concept of a biosensor is from Dr. Leland C. Clark, who worked on biosensors in the early 1960s *(2)* developing an "*enzyme electrode*" for glucose concentration measurement with the enzyme glucose oxidase, a measurement that is important in the diagnosis and treatment of disorders of carbohydrate metabolism in diabetes patients. Still today, the most common biosensors used are for glucose analysis.

A large number of basic biosensors, all combining a biological recognition element and a transducer, were subsequently developed. Currently, the trend is toward more complex integrated multianalyte sensors capable of more comprehensive analyses. Advances in electronics and microelectrical and mechanical systems (MEMS) have enabled the miniaturization of many biosensors and the newest generation biosensors include miniaturized multianalyte devices with high-throughput capabilities and more than 1,000 individually addressable sensor spots per square centimeter.

A useful categorization of biosensors is to divide them into two groups: direct recognition sensors, in which the biological interaction is directly measured, and indirect detection sensors, which rely on secondary elements for detection. **Figure 1** shows a schematic of the two groups of biosensors. In each group, there are several types of transducers including optical, electrochemical, and mechanical. For all of these technologies, the recognition ligand plays a major role. Although the most commonly used ligands are antibodies, other ligands are being developed including aptamers (protein-binding nucleic acids) and peptides.

In the literature and in practice, there are numerous types of biosensors, and the choice of a suitable system for a particular application is complex, based on many factors such as the nature of the application, the label molecule (if used), the sensitivity required, the number of channels (or area), cost, technical expertise, and the speed

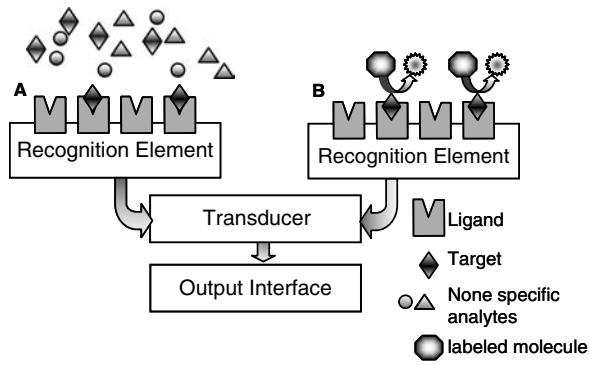

Fig. 1. General schematic of biosensors: (**a**) direct detection biosensors where the recognition element is label free; (**b**) indirect detection biosensors using a "sandwich" assay where the analyte is detected by labeled molecule. Direct detection biosensors are simpler and faster but typically yield a higher limit of detection compared with indirect detection systems

of detection needed. A primary purpose of this book is to provide more access to the technical methods involved in using a variety of biosensors to facilitate such decision making.

Direct detection biosensors utilize direct measurement of the biological interaction. Such detectors typically measure physical changes (e.g., changes in optical, mechanical, or electrical properties) induced by the biological interaction, and they do not require labeling (i.e., label free) for detection. Direct biosensors can also be used in an indirect mode, typically to increase their sensitivity. Direct detection systems include optical-based systems (most common being surface plasmon resonance) and mechanical systems such as quartz crystal resonators.

Indirect detection sensors rely on secondary elements (labels) for detection. Examples of such secondary elements are enzymes (e.g., alkaline phosphatase or glucose oxidase) and fluorescently tagged antibodies that enhance detection of a sandwich complex. Unlike direct detectors, which directly measure changes induced by biological interactions and are "label free," indirect detectors require a labeled molecule to bind to the target. Most indirect sensors based on optical detection are designed to measure fluorescence. The detection system can be based on a charge coupled device (CCD), photomultiplier tube (PMT), photodiode, or spectrometer. Electrochemical transducers, which measure the oxidation or reduction of an electroactive compound on the secondary ligand, are another common type of indirect detection sensor. Several types of electrochemical biosensors are in use including amperometric devices, which measure the electric current as a function of time while the electrode potential is held constant.

Ligands are recognition molecules that bind specifically with the target molecule to be detected. The most important characteristics for ligands are affinity and specificity. Various types of ligands are used in biosensors. Biosensors that use antibodies as recognition elements (immunosensors) are common because antibodies are highly specific, versatile, and bind strongly and stably to the antigen. Several limitations of

antibodies are long-term stability, and manufacturing costs, especially for multitarget biosensor applications where many ligands are needed.

Other types of ligands that show promise for high-throughput screening and chemical synthesis are aptamers and peptides. Aptamers are protein-binding nucleic acids (DNA or RNA molecules) selected from random pools on the basis of their ability to bind other molecules with high affinity. Peptides can be selected for affinity to a target molecule by display methods (phage display and yeast display). However, in general, the binding affinity of peptides is lower than the affinity of antibodies or aptamers.

2. Biosensor Applications

Biosensors have several potential advantages over other methods of biodetection, especially increased assay speed and flexibility. Rapid, essentially real-time analysis can provide immediate interactive information to users. This speed of detection is an advantage in essentially all applications.

Applications of biosensors include medical, environmental, public security, and food safety areas. Medical applications include clinical, pharmaceutical and device manufacturing, and research. Biosensor-based diagnostics might facilitate disease screening and improve the rates of earlier detection and attendant improved prognosis. Such technology may be extremely useful for enhancing health care delivery in the community setting and to underserved populations. Environmental applications include spill clean-up, monitoring, and regulatory instances. Public safety applications include civil and military first responders as well as unattended monitoring. Food safety applications include monitoring of food production, regulatory monitoring, and diagnosis of food poisoning. Biosensors allow multitarget analyses, automation, and reduced costs of testing.

The key strengths of biosensors are the following:

- *Fast or real-time analysis:* Fast or real-time detection provides almost immediate interactive information about the sample tested, enabling users to take corrective measures before infection or contamination can spread.
- *Point-of-care detection:* Biosensors can be used for point-of-care or on-site testing where state-of-the-art molecular analysis is carried out without requiring a state-of-the-art laboratory.
- *Continuous flow analysis:* Many biosensor technologies can be configured to allow continuous flow analysis. This is beneficial in food production, air quality, and water supply monitoring.
- *Miniaturization:* Biosensors can be miniaturized so that they can be integrated into powerful lab-on-a-chip tools that are very capable while minimizing cost of use.
- *Control and automation:* Biosensors can be integrated with on-line process monitoring schemes to provide real-time information about multiple parameters at each production step or at multiple time points during a process, enabling better control and automation of many industrial and critical monitoring facilities.

3. Aims and Approach

The primary aim of this book is to describe the basic types and the basic elements of biosensors from methods point of view. We tried to include manuscripts that represent the major technologies in the field and to include enough technical detail so that the informed reader can both understand the technology and also be able to build similar devices. The target audience for this book includes engineering, chemical, and physical science researchers, who are developing biosensing technologies. Other target groups are biologists and clinicians, who are the users and developers of applications for the technologies.

In addition to supporting the research community, the book may also be useful as a teaching tool for bioengineering, biomedical engineering, and biology faculty and students. To better represent the field, most topics are covered by more than one chapter. The purpose of this "redundancy" is to try to include several alternative approaches for the topics, so as to help the reader choose an appropriate design.

4. Chapter Organization

This publication is divided into two volumes: Vol. 503 is focused on Optical-Based Detectors and Vol. 504 is focused on Electrochemical and Mechanical Detectors, Lateral Flow, and Ligands for Biosensors.

4.1. Volume 503: Optical-Based Detectors

Optical detection is used in a broad array of biosensor technologies, including both direct and indirect style sensors. Volume 503 is organized in two parts. **Part I** focuses on direct optical detectors, while **Part II** concentrates on indirect optical detection. Probably, the most common approach for direct optical detection is based on evanescent wave physics, where the interaction between the evanescent wave and the bound target generates a detection signal. The most common technology in this group is surface plasmon resonance (SPR) and several chapters (*see* Chaps. 1–5) describe biosensors based on SPR. Other important optical direct detection methods including resonant mirror (*see* Chap. 6), optical ring resonator (*see* Chap. 7), interferometric sensors (*see* Chaps. 8 and 9) and grating coupler (*see* Chap. 10) are all included in **Part I**. The second part of Vol. 503 describes various indirect optical detectors. As discussed earlier, indirect detectors require a labeled molecule to bind to the target generating a signal. For optical sensors, the label molecule emits or modifies light. Most indirect optical detectors are designed to measure fluorescence. However, optical detectors can also measure optical density (densitometry), changes in color (colorimetric), and chemoluminesence, depending on the type of label used. Optical signals can be measured in various ways (described in **Part II**) including various CCD-based detectors, which are very versatile, inexpensive, and relatively simple to construct and use (*see* Chaps. 11–16 and 25). Other optical detectors discussed in **Part II** are photodiodes (*see* Chaps. 17–20), photomultipliers (*see* Chaps. 21–23), and spectrometers (*see* Chaps. 24 and 25). Photomultipliers may offer higher sensi-

tivity, smaller footprint (the size of photodiode can be few millimeters). Spectrometers offer better interrogation of changes in light wavelengths.

4.2. Volume 504: Electrochemical and Mechanical Detectors, Lateral Flow, and Ligands

Volume 504 describes various electrochemical and mechanical detectors, lateral flow devices, and ligands for biosensors. As in Vol. 503, we describe several direct measurement sensors (in **Part I**), indirect methods (**Parts II–III**). Ligands are described in **Part IV** and two related technologies are described in **Part V**.

In **Part I**, we describe several mechanical detectors that modify their mechanical properties as a result of biological interactions. Such mechanical direct biosensors typically sense resonance of the mechanical element, which changes when the target molecule binds to the surface. Piezoelectric biosensors (*see* Chaps. 1–3) employ a technology that is widely used in a variety of applications (e.g., vapor deposition of metals) and is thus readily available and relatively inexpensive. Cantilever-based systems (*see* Chaps. 4 and 5) can be miniaturized to micrometer dimensions with attendant benefits for system and sample size.

In **Part II**, we describe several electrochemical detectors (*see* Chaps. 6–11). Electrochemical biosensors were the first biosensors developed and are the most commonly used biosensors today (e.g., glucose monitoring).

Part III covers lateral flow technologies (*see* Chaps. 12–15). Although lateral flow devices are not "classical" biosensors, with ligands and transducers, they are included in this book because of their importance for biosensing. Lateral flow assays are simple immunodetection (or DNA hybridization) devices, which utilize competitive or sandwich assays. They are used mainly for medical diagnostics, including laboratory, home and point-of-care detection. A common format is a "dipstick" in which the test sample diffuses through a porous matrix via capillary action followed by detection by a colorimetric reagent bound to a secondary antibody. The primary antibody is bound to the matrix in a line, and the assay result is a color change at a particular location on the matrix. Lateral flow assays can be dependable and inexpensive.

Part IV focuses on recognition ligands, which are key elements in any biosensor (*see* Chaps. 16–22). The recognition ligands bind specifically with the target molecule to be detected. Various ligands described in **Part IV** include antibodies, aptamers, and peptides. Antibodies are the most commonly used ligands but advances in selection methods for aptamers (SELEX) and peptides (phage and yeast display) are currently providing alternatives.

Part V includes two papers on protein (*see* Chap. 23) and DNA preparation (*see* Chap. 24). These papers are relevant to the subject of biosensor technologies but did not fit elsewhere into the book organization outline.

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Contributors

Salvador Alegret • *Group of Sensors & Biosensors, Autonomous University of Barcelona, Barcelona, Catalonia, Spain*

Mar Álvarez • *CIBER BBN, Instituto de Microelectrónica de Madrid, Madrid, Spain*

Adriano Ambrosi • *School of Chemical Sciences, Dublin City University, Dublin, Ireland*

Antje J. Baeumner • *Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA*

Alessandra Bini • *Dipartimento di Chimica, Università degli Studi di Firenze, Sesto Fiorentino, Italy*

Kathlynn C. Brown • *Division of Translational Research, Department of Internal Medicine and Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Laura G. Carrascosa • *CIBER BBN, Instituto de Microelectrónica de Madrid, Madrid, Spain*

María Teresa Castañeda • *Nanobioelectronics & Biosensors Group, Institut Català de Nanotecnologia, Barcelona, Catalonia, Spain Group of Sensors & Biosensors, Autonomous University of Barcelona, Barcelona, Catalonia, Spain*

Paul T. Charles • *Center for Bio/Molecular Science & Engineering, US Naval R esearch Laboratory, Washington, DC, USA*

Paul Corstjens • *Leiden University Medical Center, Leiden, The Netherlands*

Alfredo de la Escosura • *Nanobioelectronics & Biosensors Group, Institut Català de Nanotecnologia, Barcelona, Catalonia, Spain*

Susan L. Deutscher • *Department of Biochemistry, University of Missouri, and Harry S. Truman Veterans Memorial Hospital, Columbia, MO, USA*

Juan E. Diaz • *Department of Chemistry, University of California, Irvine, Irvine, CA, USA*

Katie A. Edwards • *Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA*

Andrew D. Ellington • *Department of Biochemistry, University of Texas at Austin, Austin, TX, USA*

Araceli González-Cortés • *Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, Madrid, Spain*

Junqing Guo • *The Provincial Key Laboratory of Animal Immunology, Henan Academy of Agricultural Sciences, Zhengzhou, People's Republic of China*

Jan Halámek • *Department of Analytical Biochemistry, University of Potsdam, Potsdam, Germany Biophysical Engineering Group, University of Twente, Enschede, The Netherlands*

John P. Hart • *Faculty of Health and Life Sciences, University of the West of England, Bristol, UK*

Keith E. Herold • *Fischell Department of Bioengineering, University of Maryland, College Park, MD, USA*

Kagan Kerman • *Department of Chemistry, University of Western Ontario, London, ON, Canada*

David M. Kranz • *Department of Biochemistry, University of Illinois, Urbana, IL, USA*

Jorge A. Lamboy • *Department of Chemistry, University of California, Irvine, Irvine, CA, USA*

Serena Laschi • *Dipartimento di Chimica, Università degli Studi di Firenze, Sesto Fiorentino, Italy*

Laura M. Lechuga • *CIBER BBN, Instituto de Microelectrónica de Madrid, Madrid, Spain*

Shunzi Li • *Division of Translational Research, Department of Internal Medicine and Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Yingfu Li • *Department of Biochemistry and Biomedical Sciences and Department of Chemistry, McMaster University, Hamilton, ON, Canada*

Alexander Makower • *Department of Analytical Biochemistry, University of Potsdam, Potsdam, Germany*

Marco Mascini • *Dipartimento di Chimica, Università degli Studi di Firenze, Sesto Fiorentino, Italy*

Andriy Matviyenko • *Department of Engineering, University of Maryland, College Park, MD, USA FDA Center for Devices and Radiological Health, Silver Spring, MD, USA*

Michael Mauk • *School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA, USA*

Michael J. McGuire • *Division of Translational Research, Department of Internal Medicine and Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Arben Merkoçi • *Nanobioelectronics & Biosensors Group, Institut Català de Nanotecnologia, Barcelona, Catalonia, Spain*

Maria Minunni • *Dipartimento di Chimica, Università degli Studi di Firenze, Sesto Fiorentino, Italy*

Wing Ki Mok • *Department of Biochemistry and Biomedical Sciences and Department of Chemistry, McMaster University, Hamilton, ON, Canada*

Raj Mutharasan • *Department of Chemical and Biological Engineering, Drexel University, Philadelphia, PA, USA*

Naveen K. Navani • *Department of Biochemistry and Biomedical Sciences and Department of Chemistry, McMaster University, Hamilton, ON, Canada*

Jessica R. Newton • *Department of Biochemistry, University of Missouri, Columbia, MO, USA*

R. Sam Niedbala • *Department of Chemistry, Lehigh University, Bethlehem, PA, USA*
Supriya S. Pai • *Department of Microbiology, University of Texas at Austin, Austin, TX, USA*
Ilaria Palchetti • *Dipartimento di Chimica, Università degli Studi di Firenze, Sesto Fiorentino, Italy*
Roy M. Pemberton • *Faculty of Health and Life Sciences, University of the West of England, Bristol, UK*
Reginald M. Penner • *Department of Chemistry, University of California, Irvine, Irvine, CA, USA*
José M. Pingarrón • *Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, Madrid, Spain*
Jose A. Plaza • *CIBER BBN, Instituto de Microelectrónica de Madrid, Madrid, Spain*
Avraham Rasooly • *FDA Center for Devices and Radiological Health, Silver Spring, MD, USA National Cancer Institute, Bethesda, MD, USA*
Sarah A. Richman • *Department of Biochemistry, University of Illinois, Urbana, IL, USA*
Frieder W. Scheller • *Department of Analytical Biochemistry, University of Potsdam, Potsdam, Germany*
Nikolay Sergeev • *FDA Center for Devices and Radiological Health, Silver Spring, MD, USA*
Lisa C. Shriver-Lake • *Center for Bio/Molecular Science & Engineering, US Naval Research Laboratory, Washington, DC, USA*
Robert W. Siegel • *Abbott, Diagnostic Division, Abbott Park, IL, USA Eli Lilly and Company, Lilly Research Laboratories, Greenfield, IN, USA*
Petr Skládal • *Department of Biochemistry, Masaryk University, Brno, Czech Republic*
Jennifer D. Stone • *Department of Biochemistry, University of Illinois, Urbana, IL, USA*
Chris R. Taitt • *Center for Bio/Molecular Science & Engineering, US Naval Research Laboratory, Washington, DC, USA*
Eiichi Tamiya • *Department of Applied Physics, Graduate School of Engineering, Osaka University, Suita, Osaka, Japan*
Carsten Teller • *Department of Analytical Biochemistry, University of Potsdam, Potsdam, Germany*
Sara Tombelli • *Dipartimento di Chimica, Università degli Studi di Firenze, Sesto Fiorentino, Italy*
Mun'delanji Vestergaard • *School of Materials Science, Japan Advanced Institute of Science and Technology (JAIST), Nomi City, Ishikawa, Japan*
Alexander Volkov • *Department of Chemistry, Lehigh University, Bethlehem, PA, USA*
Shuo Wang • *Key Laboratory of Food Nutrition and Safety, Ministry of Education, Tianjin Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology, Tianjin, China*

Xuannian Wang • *The Provincial Key Laboratory of Animal Immunology, Henan Academy of Agricultural Sciences, Zhengzhou, People's Republic of China*

Gregory A. Weiss • *Department of Chemistry, University of California, Irvine, Irvine, CA, USA*

Paloma Yáñez-Sedeño • *Department of Analytical Chemistry, Faculty of Chemistry, University Complutense of Madrid, Madrid, Spain*

Li-Mei C. Yang • *Department of Chemistry, University of California, Irvine, Irvine, CA, USA*

Can Zhang • *Key Laboratory of Food Nutrition and Safety, Ministry of Education, Tianjin Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology, Tianjin, China*

Gaiping Zhang • *The Provincial Key Laboratory of Animal Immunology, Henan Academy of Agricultural Sciences, Zhengzhou, People's Republic of China*

Yan Zhang • *Key Laboratory of Food Nutrition and Safety, Ministry of Education, Tianjin Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology, Tianjin, China*

Kiril Zinoviev • *CIBER BBN, Instituto de Microelectrónica de Madrid, Madrid, Spain.*

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