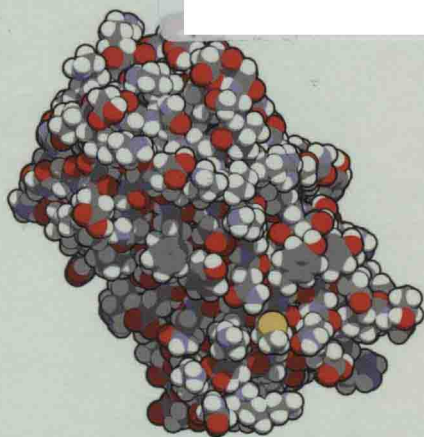
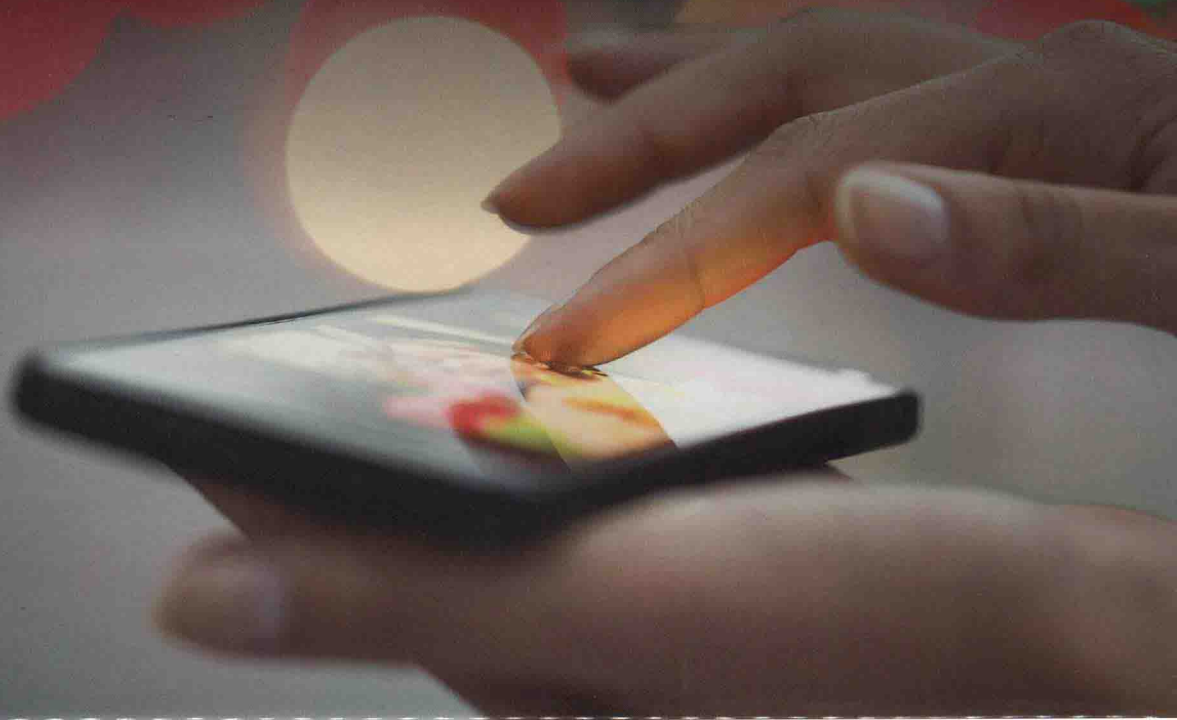




EDITED BY
CHANDRAN KARUNAKARAN, KALPANA BHARGAVA
AND ROBSON BENJAMIN



BIOSENSORS AND BIOELECTRONICS



BIOSENSORS AND BIOELECTRONICS

Edited by

CHANDRAN KARUNAKARAN

Biomedical Research Laboratory, Department of Chemistry,
VHNSN College (Autonomous), Virudhunagar, Tamil Nadu, India

KALPANA BHARGAVA

Peptide and Proteomics Division, Defence Institute of Physiology
and Allied Sciences (DIPAS), Defence Research and Development
Organisation (DRDO), Delhi, India

ROBSON BENJAMIN

Department of Physics, American College (Autonomous),
Madurai, Tamil Nadu, India



ELSEVIER

Amsterdam • Boston • Heidelberg • London • New York • Oxford
Paris • San Diego • San Francisco • Singapore • Sydney • Tokyo

Elsevier

Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK
225 Wyman Street, Waltham, MA 02451, USA

Copyright © 2015 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

ISBN: 978-0-12-803100-1

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

For Information on all Elsevier publications visit
our website at <http://store.elsevier.com/>



Working together
to grow libraries in
developing countries

www.elsevier.com • www.bookaid.org

Printed and bound in the United Kingdom

BIOSENSORS AND BIOELECTRONICS

CONTRIBUTORS

Chandran Karunakaran

Biomedical Research Laboratory, Department of Chemistry, VHNSN College (Autonomous), Virudhunagar, Tamil Nadu, India

Kalpana Bhargava

Peptide and Proteomics Division, Defence Institute of Physiology and Allied Sciences (DIPAS), Defence Research and Development Organisation (DRDO), Delhi, India

Robson Benjamin

Department of Physics, American College (Autonomous), Madurai, Tamil Nadu, India

Niroj Kumar Sethy

Peptide and Proteomics Division, Defence Institute of Physiology and Allied Sciences (DIPAS), Defence Research and Development Organisation (DRDO), Delhi, India

Mainak Das

Biological Sciences and Bioengineering, Design Program, Indian Institute of Technology (IIT), Kanpur, Uttar Pradesh, India

Surendran Elango

Department of Computer Science, VHNSN College (Autonomous), Virudhunagar, Tamil Nadu, India

Krishna Arun Venkatesh

University Science Instrumentation Centre, Madurai Kamaraj University, Madurai, Tamil Nadu, India

Manickam Pandiaraj

Biomedical Research Laboratory, Department of Chemistry, VHNSN College (Autonomous), Virudhunagar, Tamil Nadu, India

Thangamuthu Madasamy

Biomedical Research Laboratory, Department of Chemistry, VHNSN College (Autonomous), Virudhunagar, Tamil Nadu, India

Paulraj Santharaman

Biomedical Research Laboratory, Department of Chemistry, VHNSN College (Autonomous), Virudhunagar, Tamil Nadu, India

Raju Rajkumar

Biomedical Research Laboratory, Department of Chemistry, VHNSN College (Autonomous), Virudhunagar, Tamil Nadu, India

PREFACE

The aim of this book is to provide the basics of the core concepts, methods, and instrumentation of biosensors, a multidisciplinary field requiring knowledge not limited to basic science, viz., biology, chemistry, and physics but also medicine and engineering. It is hoped that the reader will acquire the fundamentals and the state-of-the-art developments in this subject as it stands today. This book is dedicated to students, researchers, and R&D scientists who wish to understand, enhance, and broaden their knowledge and expertise on the fundamentals, technologies, and applications of biosensors. The book begins with the definition of biosensors and their classifications depending upon transduction and bioreceptor components. Molecular recognition based on geometry and forces of interaction plays an important role in biosensor development. Among the biosensor techniques, electrochemical biosensors are cost-effective, miniaturized, and applied for point-of-care application studies. Hence, their theory and methods are discussed in detail. Chapter 2 presents the advent of nanotechnology for development of highly efficient nanobiosensors. Organic conducting nanopolymers in organic electronics and electrochemical transistor, inorganic nanoparticles, and their nanocomposites as excellent transducers have shown to be biocompatible. Hence, the nanocomposites provide an immobilization matrix for various bioreceptors. Chapter 3 focuses on enzymatic and their mimetic-based biosensors for the determination of various biomarkers. Several pathologies are identified by the presence of their specific markers, for instance, cytochrome c for measurement of apoptosis, nitric acid and metabolites as hypoxia markers, cholesterol for cardiovascular disease, etc. Their development, including their optimization and characteristics of biosensors for these biomarkers, has been demonstrated.

Chapter 4 presents the recent advances in antibodies research and their applications for immunosensor development. Electrochemical immunosensors for metalloproteins, nonmetalloproteins, and cancer cells using their specific antibodies are emphasized. In Chapter 5, the essential components for the development of biosensors instrumentation are discussed. Virtual electrochemical instrumentation development using labVIEW is presented. Also, hand held microcontroller-based electrochemical biosensors are assembled and applied for the measurement of various biomarkers including cytochrome c and nitric oxide metabolites. These chapters provide the essential background knowledge and up-to-date advances in this field. The book should thus serve as an introductory text for those who intend to specialize in either the theoretical or practical applications. It is hoped that this textbook will be a fruitful launch pad for many careers in biosensors and bioelectronics.

Many thanks are due to Dr Kalpana Bhargava and Dr Robson Benjamin for their constant support and cooperation as coeditors and authors of this book, all the contributing authors from a wide variety of disciplines, and the collaborators and researchers from around the globe for their achievements in this field and who have made valuable contributions that have made this book possible. It is a true pleasure to acknowledge the following: Prof. R. Murugesan, Prof. B. Kalyanaraman, and all teachers for their inspiration and guidance: Dr A. Rajendran, Dr Srigiridhar Kotamraju, Dr Shasi Kalivendi, Dr Anuradha Dhanasekaran, Dr Sushil Kumar Singh, Dr D. Christopher Durairaj, Dr S. Elango, Dr K. Arun Venkatesh, Mr K. Vairamani and all colleagues for their input and sharing knowledge; the principal and managing board of our institution for their encouragement and cooperation; Prof. George Malliaras and Dr D. Raja Shunmugam for their suggestions and critical comments; my wife and children for their perseverance and support during the preparation of the book; and Mr K. Harish for reading through the manuscript. I am also indebted to Dr S. Rajesh, Dr U.S.E. Arivudainambi, Dr S. Rajasingh, Dr S. Prakash, Dr P. Gurusamy, Dr V. Ramesh, Dr M. Pandiaraj, Dr T. Madasamy, Dr Manjulata Singh, Mr Aditya Arya, Mr M. Balamurugan, Mr G. Muneeswaran, Mr P. Santharaman and Mr R. Rajkumar for their contributions that enabled me to undertake this task and complete it. Finally, it has been a great experience to work with my publisher, Elsevier, and special thanks to them for their effort in making this book possible.

Dr Chandran Karunakaran

CONTENTS

<i>Contributors</i>	<i>ix</i>
<i>Preface</i>	<i>xi</i>
1. Introduction to Biosensors	1
Chandran Karunakaran, Raju Rajkumar, Kalpana Bhargava	
1.1 Introduction	2
1.2 Basic principle of a biosensor	3
1.3 Components of a biosensor	3
1.4 Molecular recognition	17
1.5 Classification of biosensors based on transducers	23
1.6 Piezoelectric biosensors	32
1.7 Magnetoelastic biosensors	35
1.8 Field effect transistor-based biosensor	36
1.9 Calorimetric biosensor	38
1.10 Noninvasive biosensors	38
1.11 Electrochemical biosensors	42
1.12 Various electrochemical techniques	46
1.13 Electroanalytical characteristics of biosensors	58
1.14 Membranes used in biosensors for selectivity	60
1.15 Biosensor electrode fabrication techniques	61
References	66
2. Nanocomposite Matrix Functionalization for Biosensors	69
Chandran Karunakaran, Paulraj Santharaman, Mainak Das	
2.1 Introduction	70
2.2 Organic conducting polymers	71
2.3 Inorganic nanoparticles	86
2.4 Chitosan and Nafion	116
2.5 Immobilization strategies	119
2.6 Properties of immobilized enzymes	127
2.7 The biology of enzyme immobilization	128
References	128
3. Enzymatic Biosensors	133
Chandran Karunakaran, Thangamuthu Madasamy, Niroj Kumar Sethy	
3.1 Enzymatic biosensors	135
3.2 History of biosensors	135

3.3	Enzymatic and nonenzymatic biosensors for various diseases	137
3.4	Biomarkers for diagnosis of diseases	139
3.5	Glucose oxidase-based glucose biosensors for diabetes	141
3.6	Noninvasive glucose biosensor	143
3.7	Implantable glucose biosensors	144
3.8	Cholesterol biosensor	144
3.9	Oxidative stress biomarkers	146
3.10	Superoxide anion radical biosensor	147
3.11	Thiol biosensor	151
3.12	Nitric oxide biosensor	153
3.13	Nitrite biosensor	157
3.14	Nitrate reductase-based biosensor for nitrate	158
3.15	Apoptosis marker	162
3.16	Simultaneous determination of biomarkers	168
3.17	Bienzymatic biosensor	170
3.18	Enzyme inhibition-based biosensors	171
3.19	Enzyme mimetic (metalloporphyrin)-based biosensors	173
3.20	Screen-printed functionalized electrodes and advantages	181
3.21	Nanocomposite-enhanced electrochemical biosensors	181
3.22	Recent applications	187
3.23	Veterinary	191
3.24	Food and agriculture	192
3.25	Biomedical applications	193
	References	196
4.	Immunosensors	205
	Chandran Karunakaran, Manickam Pandiaraj, Paulraj Santharaman	
4.1	Introduction	206
4.2	Antibody as biorecognition element	207
4.3	Types of antibodies and antibody fragments	208
4.4	Types of immunosensors	213
4.5	Labeled and label-free immunosensors	226
4.6	Immunosensor applications	239
4.7	Future prospects	242
	References	243
5.	Instrumentation	247
	Krishna Arun Venkatesh, Robson Benjamin, Chandran Karunakaran, Surendran Elango	
5.1	Virtual instrumentation	249
5.2	Introduction to NI LabVIEW	249

5.3	Difference between LabVIEW and conventional languages	250
5.4	Front panel	250
5.5	Block diagram	252
5.6	Icon and connector panel	253
5.7	Controls palette	253
5.8	Function palette	254
5.9	Tools palette	255
5.10	Creating, editing, wiring, debugging, and saving VIs	255
5.11	SubVIs — creating subVIs	258
5.12	Looping: for loop, while loop	258
5.13	Shift registers and sequence locals	260
5.14	Case and sequence structures	260
5.15	MyDAQ	264
5.16	Virtual electrochemical analyzer	266
5.17	Electronics of electrochemical biosensor	279
	References	315
	<i>Index</i>	319

CHAPTER 1

Introduction to Biosensors

Chandran Karunakaran¹, Raju Rajkumar¹, Kalpana Bhargava²

¹Biomedical Research Laboratory, Department of Chemistry, VHNSN College (Autonomous), Virudhunagar, Tamil Nadu, India; ²Peptide and Proteomics Division, Defence Institute of Physiology and Allied Sciences (DIPAS), Defence Research and Development Organisation (DRDO), Delhi, India

Contents

1.1	Introduction	2
1.2	Basic principle of a biosensor	3
1.3	Components of a biosensor	3
1.3.1	Bioreceptors	4
1.3.1.1	Enzyme bioreceptors: biosensors	5
1.3.1.2	Antibody bioreceptor: immunosensors	6
1.3.1.3	Nucleic acid bioreceptors	7
1.3.1.4	Aptasensors	11
1.3.1.5	Microbial biosensors	16
1.4	Molecular recognition	17
1.4.1	Binding forces and interactions involved in molecular recognition	18
1.4.1.1	Electrostatic and hydrophobic interaction	18
1.4.1.2	Covalent bonding	19
1.4.1.3	Hydrogen bonding	19
1.4.1.4	Specific interaction	19
1.4.1.5	Molecular recognition between biotin and avidin	19
1.4.1.6	Molecular recognition between protein A/G and antibody	20
1.4.2	Molecular recognition based on geometry	20
1.4.2.1	Molecular recognition by imprinting	20
1.4.2.2	Molecular imprinting process	21
1.4.3	Types of molecular recognition	23
1.4.3.1	Static molecular recognition	23
1.4.3.2	Dynamic molecular recognition	23
1.5	Classification of biosensors based on transducers	23
1.5.1	Optical detection methods	24
1.5.2	Optical biosensors	25
1.5.3	Optical label-free biosensor	25
1.5.3.1	Surface plasmon resonance-based biosensors	25
1.5.4	Fluorescence-based biosensors	28
1.5.5	Chemiluminescence-based biosensors	31
1.6	Piezoelectric biosensors	32
1.7	Magnetoelastic biosensors	35
1.8	Field effect transistor-based biosensor	36
1.9	Calorimetric biosensor	38

1.10 Noninvasive biosensors	38
1.10.1 Saliva-based sensors	39
1.10.2 Tear-based sensors	40
1.10.3 Sweat-based sensors	40
1.10.4 Breath sensors	40
1.11 Electrochemical biosensors	42
1.11.1 Introduction	42
1.11.2 Principle of electrochemical biosensors	42
1.11.3 Electrochemical cell	42
1.11.4 Microfluidic electrochemical cell	45
1.11.5 Lab-on-a-chip	45
1.12 Various electrochemical techniques	46
1.12.1 Amperometric transducers	46
1.12.2 Voltammetric transducers	46
1.12.2.1 <i>Fundamentals of cyclic voltammetry</i>	52
1.12.3 Conductometric transducers	55
1.12.4 Impedimetric transducers	55
1.12.4.1 <i>Electrochemical impedance spectroscopy</i>	56
1.12.5 Potentiometric transducers	58
1.13 Electroanalytical characteristics of biosensors	58
1.14 Membranes used in biosensors for selectivity	60
1.14.1 Ion-selective membranes	60
1.14.2 Nafion and cellulose acetate membranes	60
1.15 Biosensor electrode fabrication techniques	61
1.15.1 Screen Printing	61
1.15.2 Liquid-handling techniques	63
1.15.3 Photolithographic techniques	65
References	66

1.1 INTRODUCTION

Bioelectronics deals with the application of the principles of electronics to biology and medicine. Biosensor is a special type of bioelectronic device commonly used in bioanalysis. A sensor can be viewed as the “primary element of a measurement chain, which converts the input variable into a signal suitable for measurement.” Over the past decade, many important technological advances have provided us with the tools and materials needed to construct biosensor devices. Since the invention of the Clark oxygen electrode sensor, there have been many improvements in sensitivity, selectivity, and multiplexing capacity of modern biosensors. *Biosensor* can be defined as a compact analytical device incorporating a biological or biologically derived sensing element either integrated within or intimately associated with a physicochemical transducer. Two fundamental operating principles of a biosensor are “biological recognition” and “sensing.” Therefore, a biosensor can be generally defined as a device that consists of three basic components connected in series: (1) a biological recognition system, often called a bioreceptor, (2) a transducer, and (3) microelectronics. The basic principle of

a biosensor is to detect this molecular recognition and to transform it into another type of signal using a transducer. The main purpose of the recognition system is to provide the sensor with a high degree of selectivity for the analyte to be measured. The interaction of the analyte with the bioreceptor is designed to produce an effect measured by the transducer, which converts the information into a measurable effect such as an electrical/optical signal. According to IUPAC recommendations 1999, a biosensor is an independently integrated receptor transducer device, which is capable of providing selective quantitative or semiquantitative analytical information using a biological recognition element (Thévenot et al., 1999). The purpose of a biosensor is to provide rapid, real-time, accurate, and reliable information about the analyte of interrogation. Ideally, it is a device that is capable of responding continuously, reversibly, and does not perturb the sample. Biosensors have been envisioned to play a significant analytical role in medicine, agriculture, food safety, bioprocessing, environmental and industrial monitoring (Luong et al., 2008).

1.2 BASIC PRINCIPLE OF A BIOSENSOR

The term *biosensor* is short for *biological sensor* and is a device made up of a transducer and a biological element that may be an enzyme, an antibody, or a nucleic acid. The biological element or bioelement interacts with the analyte being tested and the biological response is converted into an electrical signal by the transducer. Every biosensor has a biological component that acts as the sensor and an electronic component that detects and transmits the signal. In other words, the biological material is immobilized and a contact is made between the immobilized biological material and the transducer. The analyte binds to the biological material to form a bound analyte, which in turn produces the electronic response that can be measured. Sometimes the analyte is converted to a product that could be associated with the release of heat, gas (oxygen), electrons, or hydrogen ions. The transducer then converts the product-linked changes into electrical signals, which can be amplified and measured. If the bioelement binds to the analyte, the sensor is called an *affinity sensor*. If the bioelement and the analyte give rise to a chemical change that can be used to measure the concentration of a substrate, the sensor is called a *metabolic sensor*. If the biological element combines with analyte and does not change it chemically but converts it to an auxiliary substrate, the sensor is called a *catalytic sensor*. Figure 1.1 represents the basic principle of biosensor.

1.3 COMPONENTS OF A BIOSENSOR

A biosensor consists of three main elements: a bioreceptor, a transducer, and a signal processing system (David et al., 2008). Biosensors can be classified by their bioreceptor or their transducer types. The classifications of biosensors based on bioreceptors and transducers are represented in Figure 1.2.

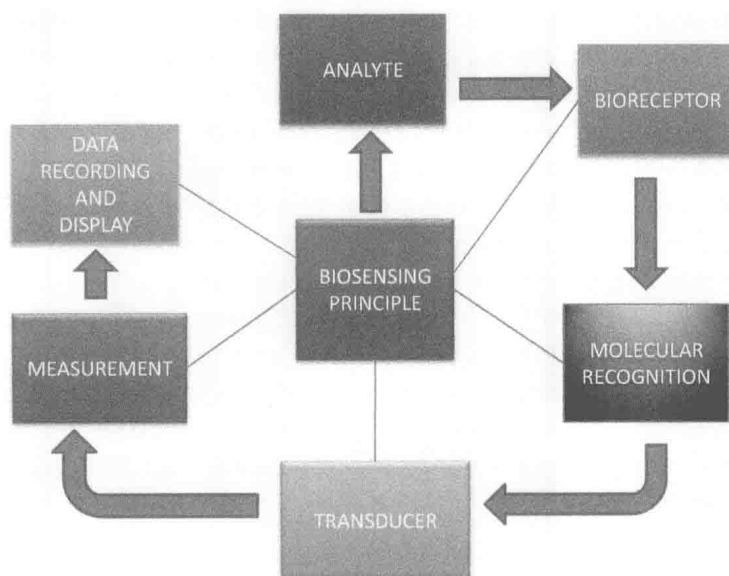


Figure 1.1 Basic principle of biosensors.

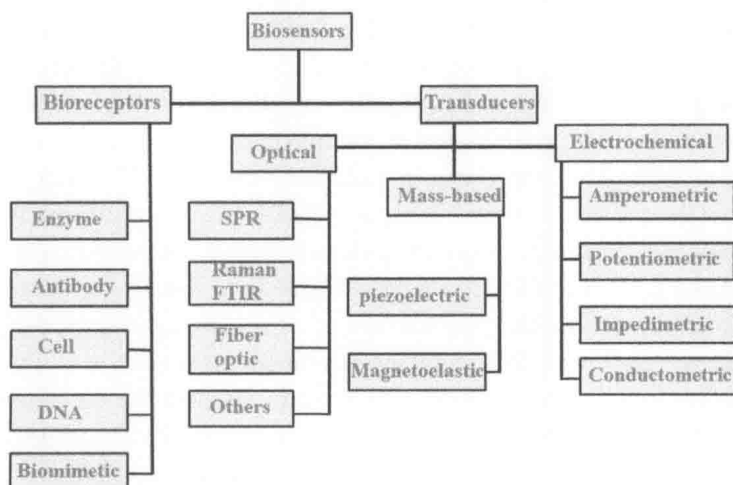


Figure 1.2 Classification of bioreceptors and transducers.

1.3.1 Bioreceptors

Bioreceptors or the biological recognition elements are the key to specificity for the biosensor technologies. The bioreceptor or biological recognition element is the significant distinguishing feature of a biosensor. The bioreceptor comprises the recognition system of a sensor toward the target analyte. A bioreceptor is molecular species that