METHODS IN MOLECULAR BIOLOGY

Series Editor
John M. Walker
School of Life and Medical Sciences
University of Hertfordshire
Hatfield, Hertfordshire, UK

For further volumes:
http://www.springer.com/series/7651

For over 35 years, biological scientists have come to rely on the research protocols and methodologies in the critically acclaimed *Methods in Molecular Biology* series. The series was the first to introduce the step-by-step protocols approach that has become the standard in all biomedical protocol publishing. Each protocol is provided in readily-reproducible step-by-step fashion, opening with an introductory overview, a list of the materials and reagents needed to complete the experiment, and followed by a detailed procedure that is supported with a helpful notes section offering tips and tricks of the trade as well as troubleshooting advice. These hallmark features were introduced by series editor Dr. John Walker and constitute the key ingredient in each and every volume of the *Methods in Molecular Biology* series. Tested and trusted, comprehensive and reliable, all protocols from the series are indexed in PubMed.

Biomimetic Sensing

Methods and Protocols

Edited by

Jessica E. Fitzgerald

Departments of Bioengineering and Chemical Engineering, Northeastern University, Boston, MA, USA

Hicham Fenniri

Departments of Chemical Engineering, Bioengineering, Chemistry and Chemical Biology, Northeastern University, Boston, MA, USA

Humana Press

Editors
Jessica E. Fitzgerald
Departments of Bioengineering
and Chemical Engineering
Northeastern University
Boston, MA, USA

Hicham Fenniri
Departments of Chemical Engineering, Bioengineering,
Chemistry and Chemical Biology
Northeastern University
Boston, MA, USA

ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-9615-5 ISBN 978-1-4939-9616-2 (eBook)
https://doi.org/10.1007/978-1-4939-9616-2

Cover illustration: Barcoded polymer-based cross-reactive sensor array response to a mixture of analytes. The different colors illustrate different responses from the sensory elements.

This Humana imprint is published by the registered company Springer Science+Business Media, LLC, part of Springer Nature.
The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Preface

The Gap in Technology: Fast, Facile, and Quantifiable Detection of Analytes in Vapor and Liquid [1, 2]

Detection of analytes for vapor and liquid deconvolution has been performed in a variety of fields, including nutrition, toxicology, biomedicine, and chemistry. The gold standard for vapor sensing is gas chromatography-mass spectrometry (GC-MS), which is a quantitative sample analysis that provides both the type and amount of analytes present in a sample, usually in the form of volatile organic compounds (VOCs). While it is advantageous to know which specific VOCs are present in a sample, GC-MS is not practical for widespread use in vapor sensing because it requires both specialized, expensive equipment and highly trained personnel for operation. In addition, some relevant data may be lost due to vapor pre-concentration and sampling techniques. Similarly, the most common liquid sensing techniques require sample labeling, or tagging, before performing an assay. This sensing technique is limited because each analyte requires a specific label (making multiplexed sensing difficult), the analytes in the sample must be known beforehand so that the correct label can be selected, and nonspecific binding may take place, affecting the accuracy of the measured analyte concentration. As sensing needs to continue to expand and develop, there remains a need for methods that enable fast, facile, and accurate sensing at a low cost. To meet these needs, many researchers have looked to the mammalian olfactory system as a model for label-free, multiplexed sensing, developing platforms that are easy to use and produce and that can be used in a wide variety of applications.

The Mammalian Olfactory System as an Optimal Model for Multiplexed Sensing

There are about 1000 genes that encode olfactory receptors (ORs), and each OR has multiple sites for odorant binding, enabling the detection of more than one odorant for each OR, a characteristic called cross-reactivity. Different combinations of activated receptors make up unique signaling codes, or "fingerprints," for specific odorants, making it possible to distinguish between thousands. This sensing platform has inspired researchers over the past several decades to develop sensing devices that are cross-reactive and accurate and have multiplexing capabilities. These biomimetic devices are called "electronic/artificial noses" (e-noses) or e-tongues to detect certain analytes present in both vapors and liquids, respectively. E-devices have proved to be successful in a broad range of scientific and engineering fields, providing cost-effective, minimally invasive (in the case of clinical use), and highly accurate vapor and liquid component analysis. In this book, we highlight the potential of e-device technology to serve as a successful platform for multiplexed sensing, along with the methods for device fabrication, calibration, and assays in multiple applications. The subsequent sections describe e-device sensing platforms, explore their use, and outline existing limitations and future directions in device development.

e-Device History and Applications

The first use of the term "electronic nose" was at a conference in 1987, and the first conference dedicated specifically to artificial olfaction was in 1989. Gardner and Bartlett originally defined an "electronic nose" as follows: "*an instrument, which comprises of an array of electronic-chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognising simple or complex odours.*" The first devices that utilized this technology were comprised of sets of distinct active materials, each connected to its own signal transduction channel. When passing an analyte vapor over the sensor array, activated sensors would transmit an electrical signal to a processor, which would then alert the user of the analytes present by cross-referencing the list of activated sensors with a database of known analyte profiles. The earliest designs employed metal oxide semiconducting field effect transistors (MOSFETs) as electrical sensors to detect gases such as NO_2. This was based on the principle that the conductivity of semiconductor metals changes upon variance in the atmospheric gas surrounding the sensor. MOSFETs are usually constructed from a SiO_2 insulating layer, with a semiconductor metal deposited on top as the gate in the circuit. A voltage is applied to maintain a constant current, and as the gas adsorbs onto the gate, the conductance of the FET changes, thereby causing the voltage to change. e-devices are able to differentiate analytes via "fingerprint" outputs; that is, each sample's analyte profile (comprised of a unique mixture of analyte types and relative concentrations) produces a unique response pattern from the sensor array, enabling sample differentiation. New sample fingerprints are compared to known sample fingerprints via data analysis (in most cases multivariate data analysis such as principle component analysis), which enables pattern recognition, clustering, and classification of the unknown analyte sample.

This fingerprint sensing method has been implemented in a number of fields, including food science, quality control, drug testing, contamination detection, defense efforts (e.g., explosive detection), and medical diagnosis. Some of the most pioneering work has been done in medical diagnostics via exhaled breath analysis. Many metabolites, or metabolic by-products, have been identified and correlated with specific diseases; some of these can even be a good indicator of disease progression. Many of these metabolites are volatile organic compounds (VOCs), which are small molecules that enter exhaled breath through gas exchange at the alveolar-capillary membrane of the respiratory tract. While the VOCs produced in each disease are thought to be primarily from oxidative stress, the subsequent effect of each disease on the body is unique and leads to the production of disease-specific VOC profiles. A reliable, noninvasive device capable of detecting subtle molecular changes and differences can be leveraged to implement this personalized medicine approach. The e-devices included in this work have much potential for implementation in these fields and others, as their sensitivity and specificity can be tuned toward specific analytes of interest.

e-Device Advances in Technological Development

Within the past several years, researchers have taken advantage of rapid technological advances to expand and improve e-device sensing platforms and sensor materials, signal transduction mechanisms, and data processing for pattern recognition and analyte identification. Moreover, as more became known about the physiology of the mammalian olfactory system, sensors further advanced to take advantage of these new findings, from

incorporating sensors that were able to detect multiple analytes to the development of a biomimetic flow chamber to enhance analyte detection at low levels. This allowed for a greater number of analytes to be recognized using a smaller array of multi-selective sensors. Within the last two decades, electronic tongues for liquid detection have also been developed, mimicking the olfactory signaling pathways.

The types of sensors employed for both vapor and liquid samples in these devices vary, including gravimetric, or mass, electrochemical, and optical sensors, allowing characterization of analytes based on mass, electrical properties (e.g., conductance impedance), and electron/photon interactive properties, respectively. Gravimetric sensors are either piezoelectric (PZ) crystals or microcantilevers, which have a specific resonant frequency. On binding with an analyte, the resonant frequency of the sensor drops in proportion to the added mass, due to either viscoelastic or gravimetric effects. Electrochemical e-devices are comprised of an electronic circuit connected to a network of sensory materials—most commonly conductive polymers or metal oxides—that provide an electrical response on binding with a specific known analyte. This response is characterized by monitoring sensor conductivity, resistivity, or voltage change during vapor exposure. Finally, optical sensors work by displaying a shift in the emission or absorption of different types of electromagnetic radiation on binding with a desired analyte. There are two popular means of optical detection: fluorescent sensors, which fluoresce upon analyte binding, and colorimetric sensors, which display a visible color change upon analyte binding.

Although gravimetric sensors have proved to be successful, there are many limitations with this device setup. Inaccuracies due to subtle changes in surface coating, humidity, or temperature necessitate frequent calibrations, which is unfortunately delicate and time-consuming. e-devices that employ optical and electrochemical sensors have shown much promise as they provide an easier and more cost-effective way of identifying analytes in vapor while maintaining accuracy. Optical sensors offer significant benefits compared with those mentioned above since they can provide multiple complex data types simultaneously, including changes in intensity, fluorescence lifetime, wavelength, and spectral shape. This approach increases the ratio of recognizable analytes to the number of sensors used. This work mainly focuses on optical (Chapters 1–10) and electrochemical (Chapters 11–13) methods of sensing, as these are at the forefront of e-device technology, being more stable and reliable than their gravimetric counterparts. We have also included a chapter on a cutting-edge mechanochemical sensing method using folded DNA origami structures (Chapter 14) that have been demonstrated to have a limit of detection down to the single molecule level. Finally, we highlight here some cutting-edge methods to optimize e-device data and technology via drift correction and calibration (Chapter 15) and computer modeling of sensor output for material optimization (Chapter 16).

Optical Sensor Arrays for e-Devices

Optical sensors in e-device systems have shown much promise to provide a facile, cost-effective, and accurate way of identifying analytes in vapor and liquid samples. Optical sensors display a shift in emission or absorption of different types of radiations upon analyte binding. The two most popular means of detection are spectroscopic and colorimetric sensing. In this work, we highlight the methods and applications of both means of detection. The spectroscopic methods employ Raman spectroscopy (Chapter 1), interferometry (Chapter 2), mass spectrometry (Chapter 3), fluorescence microscopy (Chapter 5), and

surface plasmon resonance (Chapters 8 and 10). The colorimetric sensors herein are produced in several ways, including microchip fabrication and photolithography (Chapters 4, 6, and 7). As mentioned previously, optical sensors offer significant benefits compared with gravimetric sensors; they can provide multiple complex data types simultaneously, including changes in intensity, fluorescence and colorimetric lifetime, wavelength, and spectral shape. This increases the ratio of recognizable analytes to the number of sensors used. In addition, microsphere optical arrays such as those developed by Walt et al. and presented in Chapter 1 provide an advantage over other multisensor systems because billions of beads that produce an identical response can be made simultaneously, compared with many sensors for which the fabrication process is tedious. Each type of bead has a distinct, intrinsic response to the samples presented, which eliminates the need for additional encoding for bead identification.

Electrochemical Sensor Arrays for e-Devices

Electrochemical sensing e-devices use an electronic circuit connected to a network of sensory materials that provide an electrical response upon binding with a specific known analyte. While the most common electrochemical sensing platform is the MOSFET devices, FET sensors have also been developed that incorporate organic material such as DNA-decorated graphene FETs (Chapter 13). Another popular, recent sensory material is conductive polymer (Chapters 11 and 12). Chapter 11 describes a method for layer-by-layer deposition of conducting polymers in a microfluidic channel as an electronic tongue. The conducting polymers described in Chapter 12 are electropolymerized in the presence of the target molecule or template which is then removed after polymerization to create molecularly imprinted polymers. In both cases, the polymer sensors are then placed in an electrical circuit and act as resistors, reflecting a decrease in their conductance (or an increase in impedance) upon binding with the analytes in the sample. This decrease in conductance is most likely due to polymer swelling upon analyte binding—as the polymer swells, gaps between polymer chains increase, lowering conductivity.

Remaining Challenges and Future Outlook for e-Device Implementation

As e-device implementation continues to grow in breadth, there are certain limiting factors that must be addressed. First, there remains a lack of standards for sample collection, both environmental (ambient air, water) and medical (exhaled breath, saliva). When developing a sampling method, it is important to optimize the collection, preparation, and storage method to maximize analyte detection without denaturing or altering the chemical profile of the sample. For example, collecting *alveolar* breath, the second phase of the breathing cycle, requires a sampling method that minimizes VOC interference from ambient air while capturing the alveolar air from a patient who is breathing steadily at a set velocity. The sample storage material and time of storage also affect analyte recovery. Even after obtaining an ideal sample, e-device performance accuracy may be limited by extrinsic factors such as humidity and temperature or intrinsic factors such as sensor drift and instrumentation errors. Additionally, e-device analyte fingerprint analysis via pattern recognition requires complex data analysis, which currently limits the widespread implementation of these devices.

Finally, though preliminary studies with e-devices have been largely successful, their reproducibility is limited because methods must be optimized de novo for each specific application. Standards need to be developed from statistical analysis of device performance and should include thresholds for success in areas such as response reproducibility, specificity, and sensitivity. In developing these standards, it is also important to consider the ultimate goal of the device. For example, if the goal is simply to diagnose and classify a sample, such as one that correlates with a specific disease, selectivity is more important than sensitivity; however, if the goal is to monitor analyte profile change over time, such as with disease progression, sensitivity to slight variations in analyte concentrations is of great importance.

Though these limitations currently serve as a bottleneck for widespread e-device implementation, researchers continue to work diligently to develop methods that will circumvent and overcome them. For example, Chapter 15 includes a detailed method for device calibration that can be applied to a wide variety of sensing mechanisms and e-devices. Additionally, complementary methods, such as computer modeling of analyte-sensor interaction presented in Chapter 16, serve as a way to better predict sensor response to specific analytes. This can then be leveraged to produce devices that are highly tuned toward a specific analyte or group of analytes, i.e., much improved sensitivity and selectivity. As the technology for e-device sensing continues to trend toward portable, accurate, and easy-to-use platforms, they have great potential to be implemented wherever analyte detection is required. Indeed, they may be able to reduce the need for highly specialized, expensive equipment and personnel. For developing countries in particular, e-devices that are simultaneously inexpensive may soon be able to take the place of highly specialized equipment in fields such as defense efforts and explosive detection, water and food contamination, and personalized medicine.

Boston, MA, USA

Jessica E. Fitzgerald
Hicham Fenniri

References

1. Fitzgerald JE, Bui ETH, Simon NM, Fenniri H (2016) *Trends Biotechnol.* 1
2. Fitzgerald JE, Fenniri H (2016) *RSC Adv.* 6(84): 80468

Contents

Contributors

Nageh K. Allam • *Energy Materials Laboratory, School of Sciences and Engineering, The American University in Cairo, New Cairo, Egypt*

Maria L. Braunger • *Department of Applied Physics, "Gleb Wataghin" Institute of Physics (IFGW), University of Campinas—UNICAMP, Campinas, SP, Brazil*

Collin J. Bright • *U.S Army Combat Capabilities Development Command, C5ISR Center, Fort Belvoir, VA, USA*

Olalla Calvo-Lozano • *Nanobiosensors and Bioanalytical Applications Group (NanoB2A), Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Barcelona, Spain; Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Barcelona, Spain*

Daniel Citterio • *Faculty of Science and Technology, Department of Applied Chemistry, Keio University, Yokohama, Japan*

Cristiane M. Daikuzono • *Centro de Ciências e Tecnologias para Sustentabilidade (CCTS), Universidade Federal de São Carlos—UFSCar, Sorocaba, SP, Brazil*

Adil Denizli • *Department of Chemistry, Hacettepe University, Ankara, Turkey*

Mari C. Estévez • *Nanobiosensors and Bioanalytical Applications Group (NanoB2A), Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Barcelona, Spain; Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Barcelona, Spain*

Hicham Fenniri • *Departments of Chemical Engineering, Bioengineering, Chemistry and Chemical Biology, Northeastern University, Boston, MA, USA; Department of Bioengineering, Northeastern University, Boston, MA, USA*

Jessica E. Fitzgerald • *Department of Bioengineering and Department on Chemical Engineering, Northeastern University, Boston, MA, USA*

M. Teresa S. R. Gomes • *Chemistry Department, University of Aveiro, Aveiro, Portugal; CESAM, University of Aveiro, Aveiro, Portugal*

Vera L. V. Granado • *Chemistry Department, University of Aveiro, Aveiro, Portugal*

Yuma Ikeda • *Faculty of Science and Technology, Department of Applied Chemistry, Keio University, Yokohama, Japan*

A. T. Charlie Johnson • *Department of Physics and Astronomy, University of Pennsylvania, Philadelphia, PA, USA*

Sagun Jonchhe • *Department of Chemistry and Biochemistry, Kent State University, Kent, OH, USA*

Dmitry Kirsanov • *Institute of Chemistry, St. Petersburg State University, St. Petersburg, Russia; Laboratory of Artificial Sensory Systems, ITMO University, St. Petersburg, Russia*

Laura M. Lechuga • *Nanobiosensors and Bioanalytical Applications Group (NanoB2A), Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Barcelona, Spain; Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Barcelona, Spain*

Andrey Legin • *Institute of Chemistry, St. Petersburg State University, St. Petersburg, Russia; Laboratory of Artificial Sensory Systems, ITMO University, St. Petersburg, Russia*

Fengyu Li • *Key Laboratory of Green Printing, Institute of Chemistry, Chinese Academy of Sciences (ICCAS), Beijing Engineering Research Center of Nanomaterials for Green*

Printing Technology, Beijing National Laboratory for Molecular Sciences (BNLMS), Beijing, P. R. China

Nathan C. Lindquist • *Department of Physics and Engineering, Bethel University, St. Paul, MN, USA*

Larisa Lvova • *Laboratory of Artificial Sensory Systems, ITMO University, St. Petersburg, Russia; Department of Chemical Science and Technologies, University "Tor Vergata", Rome, Italy*

Hanbin Mao • *Department of Chemistry and Biochemistry, Kent State University, Kent, OH, USA*

Takamichi Nakamoto • *Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Kanagawa, Japan*

James W. Needham • *InBios International Inc., Seattle, WA, USA*

Yuji Nozaki • *Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Kanagawa, Japan*

Vitaly Panchuk • *Institute of Chemistry, St. Petersburg State University, St. Petersburg, Russia; Laboratory of Artificial Sensory Systems, ITMO University, St. Petersburg, Russia*

Enelia C. Peláez • *Nanobiosensors and Bioanalytical Applications Group (NanoB2A), Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Barcelona, Spain; Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Barcelona, Spain*

Jinglei Ping • *Department of Mechanical and Industrial Engineering, University of Massachusetts Amherst, Amherst, MA, USA*

Alejandro Portela • *Nanobiosensors and Bioanalytical Applications Group (NanoB2A), Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Barcelona, Spain; Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Barcelona, Spain*

Meng Qin • *Key Laboratory of Green Printing, Institute of Chemistry, Chinese Academy of Sciences (ICCAS), Beijing Engineering Research Center of Nanomaterials for Green Printing Technology, Beijing National Laboratory for Molecular Sciences (BNLMS), Beijing, P. R. China*

Isabel S. Rich • *Department of Physics and Engineering, Bethel University, St. Paul, MN, USA*

Antonio Riul Jr • *Department of Applied Physics, "Gleb Wataghin" Institute of Physics (IFGW), University of Campinas—UNICAMP, Campinas, SP, Brazil*

Alisa Rudnitskaya • *Chemistry Department, University of Aveiro, Aveiro, Portugal; CESAM, University of Aveiro, Aveiro, Portugal*

Vincent P. Schnee • *U.S Army Combat Capabilities Development Command, C5ISR Center, Fort Belvoir, VA, USA*

Spencer T. Seiler • *Department of Physics and Engineering, Bethel University, St. Paul, MN, USA*

Valentin Semenov • *Institute of Chemistry, St. Petersburg State University, St. Petersburg, Russia*

Gülsu Şener • *Department of Chemistry, Hacettepe University, Ankara, Turkey*

Icell M. Sharafeldin • *Energy Materials Laboratory, School of Sciences and Engineering, The American University in Cairo, New Cairo, Egypt*

Hiroyuki Shibata • *Faculty of Science and Technology, Department of Applied Chemistry, Keio University, Yokohama, Japan*

Yanlin Song • *Key Laboratory of Green Printing, Institute of Chemistry, Chinese Academy of Sciences (ICCAS), Beijing Engineering Research Center of Nanomaterials for Green Printing Technology, Beijing National Laboratory for Molecular Sciences (BNLMS), Beijing, P. R. China*

M. Selim Ünlü • *Department of Biomedical Engineering, Boston University, Boston, MA, USA; Department of Electrical and Computer Engineering, Boston University, Boston, MA, USA*

Nese Lortlar Ünlü • *Department of Biomedical Engineering, Boston University, Boston, MA, USA*

Celalettin Yurdakul • *Department of Electrical and Computer Engineering, Boston University, Boston, MA, USA*