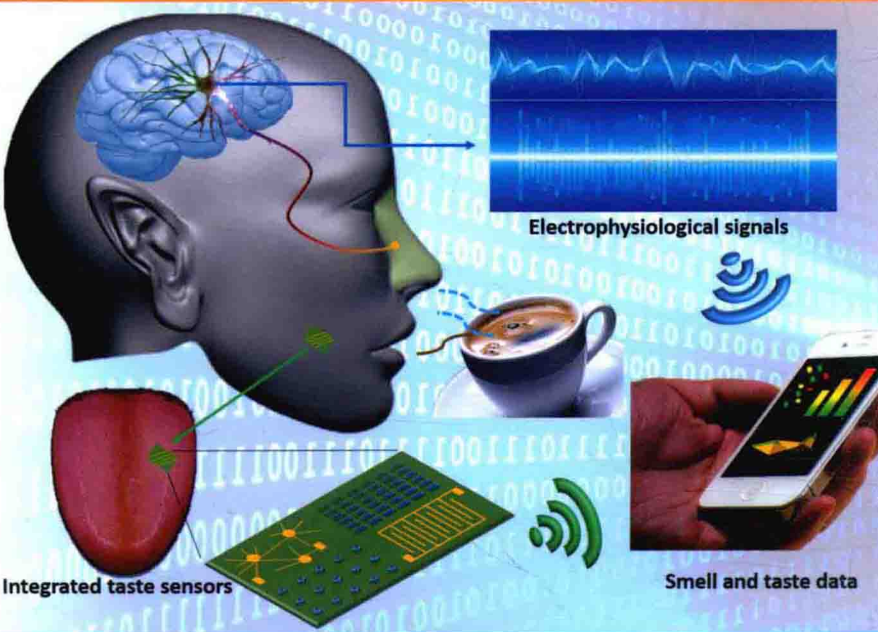

Bioinspired Smell and Taste Sensors

仿生嗅觉与味觉传感技术

Ping Wang Qingjun Liu Chunsheng Wu K. Jimmy Hsia
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Preface

The integration of cells/molecules with micro/nano devices for the development of novel sensors with unique functions has attracted intensive interest and substantial research efforts in recent decades. Exciting progress has been achieved due to the combination of micro/nano fabrication technologies with biotechnologies, which introduced new concepts and scientific paradigms to this area. Fast advancements in micro/nano structured devices are providing unprecedented opportunities for them to couple with functional cells/molecules for the development of next generation of cell and molecular sensors. Micro/nano cell and molecular sensors have become increasingly important and found wide applications in a range of areas.

Recently, an impressive number of inventive designs and technological advances for micro/nano cell and molecular-based sensing have been made that greatly contribute to their potential practical applications. These types of micro/nano cell- and molecular-based sensors combine cells/molecules with micro/nano transducers to produce a biosensor and promise to provide a simple, accurate and inexpensive platform for various applications. However, the underlying mechanisms of micro/nano cell- and molecular-based sensing are still not fully clarified, which has become one of the main challenges for their further development and improvement. Considerable basic research on the cellular and molecular sensing mechanisms as well as the coupling of cells and molecules with micro/nano devices is still highly essential and favorable for the sake of practical applications and possible commercialization.

This book summarizes the development and implementation of micro/nano cell and molecular-based sensors. The organization of this book is based on the basic concepts and potential applications of micro/nano cell- and molecular-based sensors. In addition, this book attempts to introduce the key aspects and the future perspectives of micro/nano cell and molecular sensors, especially, the cell and molecular-based biosensors with electric cell–substrate impedance sensing (ECIS), microelectrode arrays (MEAs), light-addressable potentiometric sensor (LAPS), and field-effect transistors (FETs). Throughout the book, in every chapter, the most important and recent developments relevant to the subject matter are introduced.

This book focuses on cell- and molecular-based sensors using micro/nano devices as transducers. The definition, characteristics, type, and application of micro/nano cell and molecular sensors are introduced in this book. For living cell analysis, common micro/nano cell sensors are discussed to monitor the intra- and extracellular physiological signals, including the principle, design and fabrication, application. The two main cell sensors, ECIS- and MEA-based sensors are detailed on their cell impedance and potential study, respectively. For neurons study, neural network-based sensors are focused, including the formation of neural networks on solid surface and their chemical sensing. The book is also devoted to micro/nano molecular sensors and their applications. For molecular analysis, a label-free DNA field-effect device is presented for for DNA sensing and application. Micro/nano electrochemical sensors are described for ion sensing and measurement, which is applied in field environment and food analysis. Moreover, the basic structures and properties of micro/nano material are also introduced for recent development and application in biomedicine and food analysis. At the end of this book, the future trends of micro/nano cell and molecular sensors is prospected to establish the micro/nano electromechanical cell/molecular system and Intelligent biosystem for biomimetic devices, health care, and rehabilitation.

The topics covered by this book provide a comprehensive summary of the current state of micro/nano cell and molecular sensors as well as their future development trends, which would be of great interest to the interdisciplinary community active in this area. This book is also suitable to be a comprehensive perspective for the scientists and engineers in a wide range of areas. In addition, this book could inspire and attract more and more researchers and scientists, especially the young scientists, to this area to further advance the development of micro/nano cell and molecular sensors and broaden their application fields.

Hangzhou, Ping Wang
June 2015

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Chapter 1
Introduction

Ping Wang, Qingjun Liu, Chunsheng Wu and K. Jimmy Hsia

1.1 What Are Smell and Taste Sensors

Biological olfactory and taste systems are natural chemical sensing systems that are crucial for almost all the creatures to sensing the chemical signals for various purposes such as survival, feeding, and breeding [1–7]. After long-term evolution, biological chemical sensing systems are able to detect and discriminate a large amount of chemical substances in complex environments with extreme high performances that currently cannot be matched by artificial devices. Functional components of olfactory and taste systems include olfactory and taste receptors/cells/tissues, which can be considered as natural olfactory and taste sensors because they can detect and transduce chemical signals presented by various odorants and tastants into biological signals such as neuronal action potential changes and releasing of neurotransmitters [8–10]. Inspired by the biological mechanisms of natural chemical sensing systems, different kinds of bioinspired smell and taste sensors have been developed by the combination of biological functional components for chemical sensing with various transducers [11–15]. Due to the unconventional utilization of biological mechanisms and functional components, bioinspired smell and taste sensors are characterized with high performances for chemical sensing and biochemical analysis, which exhibit high sensitivity, rapid response, and excellent selectivity.

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With the advances in biological mechanisms of olfactory and taste sensation, bioinspired smell and taste sensors have recently achieved significant progress, which have shown promising prospects and potential applications in many fields from basic research to industry. For example, the discovery of the gene family encoding vertebrate olfactory receptors (ORs) not only advances the basic research on olfactory mechanisms, but also promotes the development of bioinspired smell sensors [2]. As a result, various bioinspired smell sensors have been reported, in which biological functional components, such as olfactory tissues, olfactory sensory neurons, and olfactory receptors, have been utilized as sensitive elements for the detection of various chemical compounds [11, 15]. In addition, a non-linear neuronal network model, that is K serial models, was proposed based on the firing properties of neurons and neuronal networks at different anatomical levels of olfactory systems [16]. This model is based on the theory of nerve cell groups, which assumed that nerve cell groups formed by a similar set of cells with similar functions and features can be used as building blocks of the nervous system. On the other hand, the progress in the taste sensing mechanisms also promoted the development of bioinspired taste sensors. The discovery of important role of taste cell membrane in taste sensing [17] inspired the researchers to employ lipid-polymeric membranes that usually consist of differently charged lipids for capturing various tastant molecules [18–20].

The conventional standard methods for gas or liquid-phase detection and analysis usually rely on the utilization of precision laboratory artificial instrument like gas chromatography–mass spectrometry (GC-MS) [21]. These methods provide an accurate approach for the analysis of type and concentration of the single component in a mixture of substances. However, these methods are time-consuming, labor intensive, and expensive. The bioinspired smell and taste sensors provide novel solutions to overcome these drawbacks. In 1982, a novel gas sensing system, electronic nose (e-nose), was proposed by imitating the sensing mechanisms of biological olfactory systems [22]. In 1990, the first liquid analytical instruments based on non-specific taste sensor array—electronic tongue (e-tongue)—was reported [23]. An e-tongue using ion selective electrode array and pattern recognition algorithm was developed, which consists of a cross-sensitive chemical sensor array and pattern recognition algorithm and can be used to detect, analyze, and identify the complex chemical compositions [24]. After nearly 30 years of development, the e-noses and e-tongues have been applied in a large number of fields including food processing, environmental monitoring, public safety, and medical diagnostics.

However, limited by the development of sensor technology, the sensitivity, selectivity, and response speed of current e-noses and e-tongues are still far from that of biological olfaction and taste systems. To overcome the development bottleneck of traditional e-noses and e-tongues, in the in recent years, the concept of bioinspired smell and taste sensors have been increasing recognized as a novel approach. Figure 1.1 shows the differences in chemical sensing process by biological noses and tongues or electronic noses and tongues, which shows how the chemical signals of Brasil coffee can be detected and recognized. Unlike the traditional e-nose and e-tongues, bioinspired smell and taste sensors mimic the biological chemical sensing mechanisms and could achieve a similar performance to

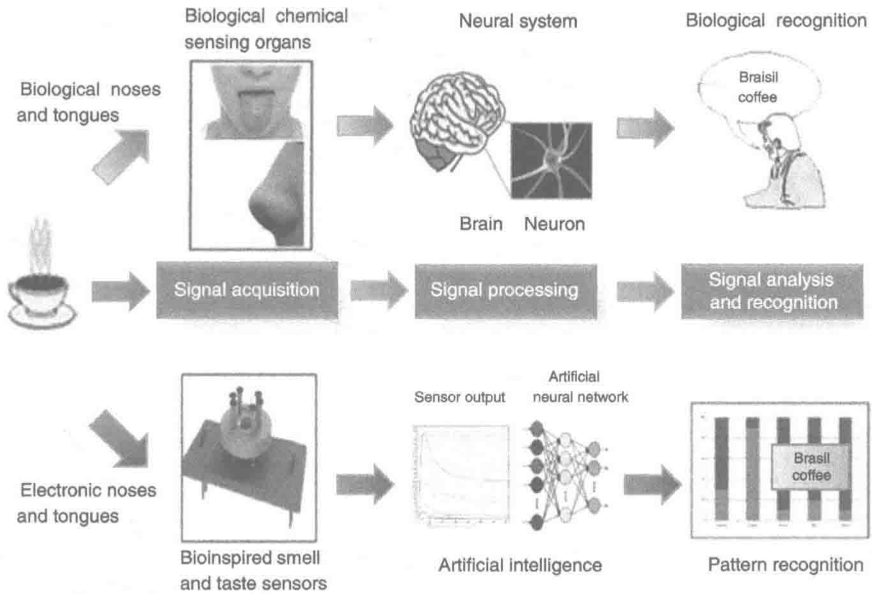

Fig. 1.1 Schematic diagram shows the comparison of biological noses and tongues with electronic noses and tongues for chemical sensing

biological olfactory and taste systems by the using of biological functional components for chemical sensing, which show prominent advantages such as high sensitivity, low detection limit, and excellent selectivity. Hence, a more broad application prospects could be expected.

1.2 Characteristics of Smell and Taste Sensors

Bioinspired smell and taste sensors consist of two main components: one is the biological functional components utilized as sensitive elements that can interact with target molecules and ions and generate the specific responses [11–15]. The other one is the physicochemical detector served as the transducer which can convert the responsive signals generated by the sensitive elements into physical signals that are easier to handle and analyze, such as electrical signals. As shown in Fig. 1.2, the composition of a complete detection system include the bioinspired sensors and the electronics and instrumentation for signal processing and display, which enable the display of detected signals in a more friendly manner. Due to the incorporation of biological functional components for chemical sensing, the biomimetic systems partly inherit the unique advantages of biological chemical sensing system such as rapid response, high sensitivity, and excellent selectivity.

At present, biological functional components used for the development of bioinspired smell and taste sensors include olfactory/taste tissues and cells,

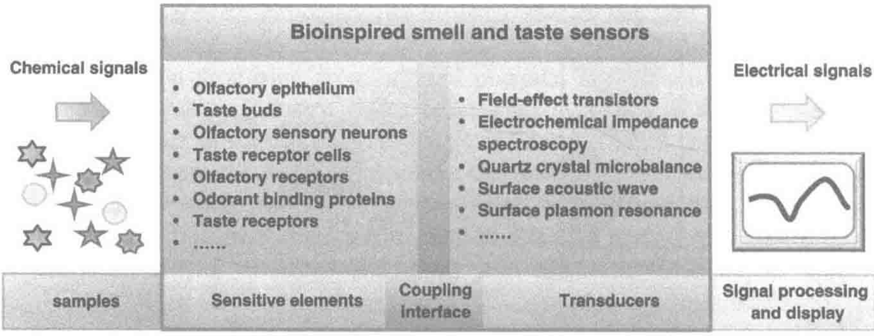

Fig. 1.2 Schematic diagram of composition and mechanisms of bioinspired smell and taste sensors for chemical sensing

olfactory/taste receptors and enzymes that can bind to the specific odorant or tastant molecules. Olfactory/taste-related proteins, such as the olfactory/taste receptor protein, and olfactory binding protein (olfactory binding proteins, OBPs), have been widely applied for the development of bioinspired smell and taste sensors due to their unique capability of binding to the specific target molecules [11]. Compared with tissues and cells, olfactory/taste receptor proteins have advantages of more easily storage for a long term, better stability, and activity, which contribute to the miniaturization and portability of bioinspired smell and taste sensors. On the other hand, although bioinspired taste sensors that utilize taste receptors as sensitive elements have achieved great progress, the current research is still at the cellular level, in which primary taste receptor cells or cell lines transfected with taste receptor genes are employed as sensitive elements for chemical sensing. Purification technology still cannot isolate purified taste receptors, which has greatly restricted the application of taste receptor proteins in bioinspired taste sensors. Bioinspired smell and taste sensors can be divided into two categories based on the detection mechanisms of different secondary sensors used transducers, which are electrical/optical detection and mass detection, respectively [11–15]. For electrical/optical detection class, conformational changes originated from the specific interactions between receptors and ligands cause the changes in the electrical/optical parameters, which can be detected by the various electrical/optical detection transducers such as field-effect transistors (FETs), microelectrode array (MEA), electrochemical impedance spectroscopy (EIS), and surface plasmon resonance (SPR). For mass detection class, the specific binding of receptors and ligands generate the mass changes that can be detected by the mass-sensitive devices such as quartz crystal microbalance (QCM) devices and surface acoustic wave (SAW) devices. The key issues for the development of bioinspired smell and taste sensors include (1) the preparation of functional biological components that can be used as sensitive elements for chemical sensing and (2) the effective coupling of sensitive elements with transducers. Based on olfactory receptors in the development of bionic type smell/taste sensor, key issues include the effective coupling preparation and olfactory receptor protein functional olfactory receptor proteins and secondary sensors fixed. These issues have direct influence on the sensitivity, specificity, and stability of bioinspired smell and taste sensors.

1.3 Types of Smell and Taste Sensors

1.3.1 Electronic Nose and Electronic Tongue

Odorant commonly contains a series of functional chemical groups and some of them serve as ligands to combine with the specific receptors. The interactions between the odorant molecules and olfactory receptors exert combinatorial effects which are specific in the epithelium. Therefore, biological olfactory system has high sensitivity and specificity to perceive and discriminate a large number of odors in the environment. For great application potential in wide fields ranging from environmental monitoring to medical diagnosis, researchers have implemented relevant investigations and designed some electronic noses, depending on absorbability or catalysis properties of sensitive materials for specific odors. However, the sensitive materials seldom performed so perfectly as the biological olfaction which owns intact structure and function of cell population in the olfactory system with the native information coding.

1.3.2 Olfactory and Taste Cell-Based Biosensors

Cell-based biosensors, which treat living cells as sensing elements, can detect the functional information of biologically active analytes. Therefore, utilizing olfactory neurons and taste cells as sensitive materials to develop bioelectronic nose and bioelectronic tongue is one of independent trends concerning the research and development of electronic nose and electronic tongue, which makes use of biomolecular function units to develop highly sensitive sensors. Some experiments, such as insect antenna and human embryonic kidney-293 cells-based biosensors, have been tried and obtained high specificity and sensitivity to drugs or odors. However, the tissue or cells were not olfactory neurons or taste cells, and the parameters detected by those sensors were also not the action potentials of the cells. Therefore, a satisfactory bioelectronic nose or bioelectronic tongue should be a hybrid system of olfactory or taste cells and extracellular potential detection transducers.

1.3.3 Olfactory and Taste Epithelium-Based Biosensors

In intact epithelium, it is possible to estimate the electrochemical potential by keeping the neuronal membrane and environment intact after the epithelium surgically removed. Whereas cells were maintained in their native environment, the acute

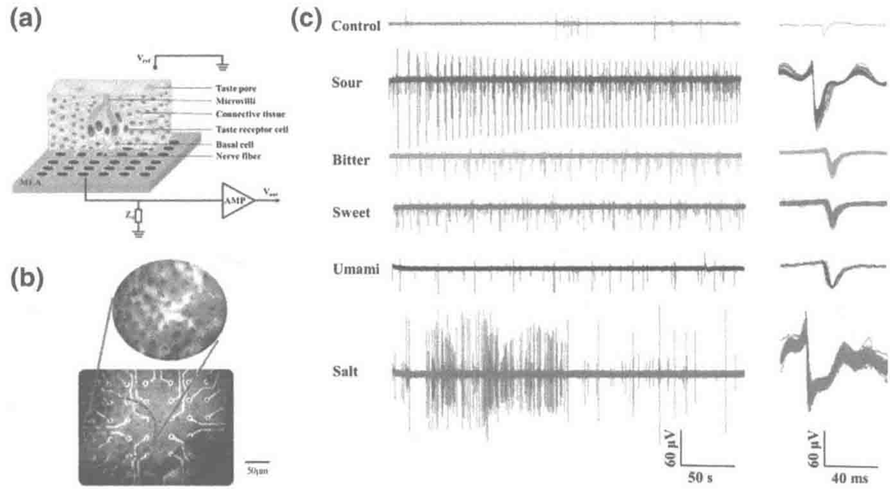

Fig. 1.3 A novel bioinspired taste sensor based on MEA chips. **a** Schematic plot of the bioinspired taste sensor composited by MEA and tongue epithelium accompanied with taste buds; **b** Microscope photograph of a MEA chip attached by tongue epithelial cells; **c** Extracellular potential of tongue epithelial cells stimulated by distinct tastants. (Reproduced with permission from Ref. [25]. Copyright 2013 Elsevier)

prepared intact epithelium had several advantages to the organotypic function over isolated olfactory receptor neurons or taste cells culture for bioelectronic nose and bioelectronic tongue: (a) the natural states of the neuronal populations of olfactory or taste receptor cells were well preserved. (b) The functional receptor unit of cilia on each receptor cell would not be damaged. (c) Extracellular compartments present *in vivo* (including supporting cells and basal cells) were preserved. (d) The mucus layer with odor binding protein generated by Bowman's glands and supporting cells were preserved in the olfactory system. (e) The intact epithelium allowed simpler acute preparation and easier visualization, without strictly controlled cell culture conditions (i.e., nutrient media, pH, temperature, and listerize). Figure 1.3 shows the schematics of a novel bioinspired gustation sensor based on MEA chips and the detected signals in responses to different taste stimuli [25].

1.3.4 Insect Antenna and Binding Proteins Smell Sensors

In insects, the first event in odors detection is the capture of the molecules by some extracellular proteins and membrane-bound olfactory receptors. One kind of the major peripheral olfactory proteins in the reception of odorants is OBPs. The proteins involved as ligand selectors and transporters for triggering the olfactory signal transduction by binding the small hydrophobic molecules to enhance their aqueous solubility and transport them to specific olfactory receptors. As selective peripheral signal filters to the actual receptor proteins, OBPs could be used as promising sense materials for a new generation of olfactory sensors with their increasing clear sensing properties. OBPs are robust enough to stand up to wide ranges of pH and temperatures for substantial mistreatments, without denaturing and losing their binding properties. In addition, OBPs are easier to be isolated and purified in the process of production compared with membrane protein of olfactory receptors. All of these will greatly enhance the practicability of those materials using in sensors.

1.3.5 Smell and Taste Receptor-Based Biosensors

The most fundamental elements are smell and taste receptors in the cilia of smell and taste sensory neurons, which contribute greatly to the high-performance smell and taste system. The excellent properties of smell and taste receptors are generally recognized in the development of biomimetic smell and taste receptors-based biosensors. Over the past two decades, much work has been done in developing smell and taste receptors based biosensors due to their promising potential in many applications. The production of functional receptors is one of the most crucial factors in the development of smell and taste receptors-based biosensors, which should maintain their natural structures and native functions to recognize their natural ligands, low production costs, and ease of storage and long-shelf life.

1.3.6 Biomimetic Membrane-Based Taste Biosensors

This kind of taste biosensors is equipped with multichannel electrodes using a lipid/polymer membrane for the transducer. The sensor is considered to be an electronic tongue with global selectivity, which is defined as the decomposition of the characteristics of a chemical substance into those of each type of taste and their quantification, rather than the discrimination of individual chemical substances, by mimicking the human tongue, on which the taste of foods is decomposed into each type of taste by each taste receptor. The taste sensor is commercialized taste sensing systems SA 402B and TS-5000Z, which are the world's first commercialized electronic tongue system and are currently well known to be able to discriminate and quantify tastes. A lipid/polymer membrane comprising a lipid, polyvinyl chloride, and a plasticizer is used for the stage of receiving taste substances, the key technology of the taste sensor.

1.3.7 In Vivo Smell and Taste Biosensing System

The *in vitro* cultured environment leads to shortened cell/tissue survival, so the working life of biosensor is short. *In vitro* culture would also damage the intact nerve structure of olfactory and taste system and the natural pattern of neuronal activity may be changed. Multiple microelectrode implant technology could overcome these limitations by investigating electrophysiological properties of neuronal populations *in vivo*. Although this technology causes local tissue damage, it ensures integrity of biological nerve system. The propensity for multi-electrode electrophysiological investigation of neuronal populations is well established in both sensory and motor systems. For the *in vivo* biosensing system has characteristic of high sensitivity, continuous recording, and specificity, it presents a promising platform for specific trace odorant and tastants detection in real application (Fig. 1.4).

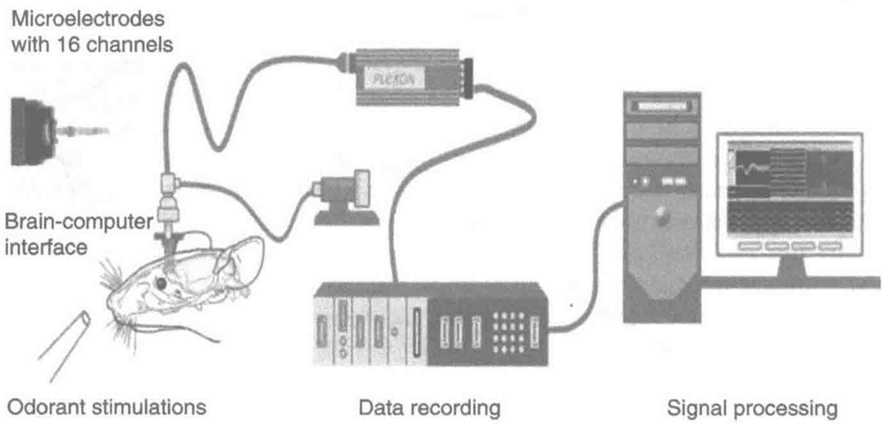

Fig. 1.4 Sketch map of bioinspired smell sensor based on implanted electrodes

1.4 Application of Smell and Taste Sensors

The biological olfactory and taste systems can recognize and discriminate a large number of environmental chemical compounds presented by odorants or tastants with amazing performances. Inspired by the biological mechanisms of olfaction and taste systems for chemical sensing, many kinds of olfaction and taste sensors have been developed by the combination of biological functional components related to olfaction and taste with various secondary sensors mainly including QCM devices, SAW devices, FETs, microelectrode, SPR devices, and light-addressable potentiometric sensor (LAPS) [11–13, 15, 26, 27]. The biological functional components are usually utilized as the sensitive materials for the development of olfaction and taste sensors, which are mainly originated from the biological olfactory and taste systems at the tissue level, cellular level, or molecular level. Similar to biological chemical sensing systems, the olfaction and taste sensors are characterized with high sensitivity, rapid response, and excellent specificity, which have shown great potential commercial prospects and promising applications in many fields such as biomedicine, environmental protection, pharmaceutical screening, and the quality control of food and water. In addition, the olfaction and taste sensors also provide novel approaches for the research of olfactory and taste signal transduction, which contribute greatly to the understanding of biological chemical sensing mechanisms. On the other hand, conventional electronic noses and electronic tongues also attracted more and more interests and have achieved significant progress in the recent decades due to their powerful chemical sensing capabilities and promising potential applications. Electronic noses and electronic tongues are usually equipped with olfaction and taste sensor arrays that can respond to diverse chemical compounds. It is thus possible to apply electronic noses and electronic tongues in the discrimination of special chemical combinations or patterns in a complex chemical environment, which can greatly broaden the application fields of olfaction and taste sensors.

1.4.1 Detection of Chemical Compounds

The most common applications of olfaction and taste sensors are connected with their capability of chemical compound detection with extreme high sensitivity and specific, which are highly essential and favorable in a wide range of fields related to the monitoring of specific chemical markers. The typical application fields include the agriculture, food safety, public safety, and anti-terrorism. In the agriculture field, an antenna-based olfaction sensor has been developed for the detection of (Z)-3-hexen-1-ol, which is a volatile chemical marker for plant damage [28, 29]. This olfaction sensor have been used to detect the plant damage in a glasshouse under real-world conditions, which can successfully distinguish single mechanically or beetle-damaged plants in background emissions of 1000 undamaged plants. In the field of food safety, an olfaction sensor have been developed based on the QCM device and odorant binding protein for the detection of volatile organic compounds indicative to *Salmonella* contamination in packaged beef, which can respond to 3-methyl-1-butanol and 1-hexanol with the detection limit as low as 5 ppm [30]. In the field of public safety and anti-terrorism, different kinds of olfaction sensors have been developed based on various chemicals sensing mechanisms for the detection of volatile chemical indicators of explosive materials as well as illicit substances, which have shown extreme high sensitivity and practical application potentials [31–34]. The taste sensors have also shown potential applications in these fields, especially in the fields of food safety. Various taste sensors have been developed, which can be used for the detection of specific tastants such as sour, sweet, and bitter substances [35–38]. These taste sensors can be used not only for the food safety monitoring, but also for the quality control of the food tasty that could have great influences on the consumption of foods. The olfaction and taste sensors have shown promising application prospects in these fields, but the research and development are still in the early experimental stage and no commercialized olfaction and taste sensors can be found in the market. This also provides opportunities for the further development of olfaction and taste sensors to meet the special requirement of practical applications.

1.4.2 Research of Signal Transduction Mechanisms

Another important application field of olfaction and taste sensors is related to the basic research of biological mechanisms of olfactory and taste systems for the detection of environmental chemical signals. In this field, olfaction and taste sensors are usually employed as promising alternative and useful tools or platforms for the further investigation of olfactory and taste signal transduction mechanisms, such as the identification of specific ligand-receptor pairs, the characterization of cellular physiology, and the electrophysiological recording of cells or tissues, which can the thus promote the research on biological olfactory and taste systems. For example, olfaction and taste sensors based on LAPS chip have been developed for the research of olfactory and taste signal transduction mechanisms at the tissue and cellular level, which can realize non-invasive, long term, and convenient measurements. An olfaction sensor developed on the basis of LAPS measurement setup has been utilized to record the membrane potential changes of rat olfactory sensory neurons (OSNs) to investigate the inhibitory effect of MDL12330A as well as the enhancive effect of LY294002 on the olfactory signals of OSNs [39]. In addition, based on the enhancive effect of LY294002 on the olfactory signals of OSNs, a novel strategy for the response enhancement of olfaction sensors was proposed, which indicate the using of LY294002 can enhance the sensitivity of olfaction sensors by 1.5 fold [40]. LAPS chip has been used as a novel platform to record the electro physiology property of living taste receptor cells in response to the five basic taste substances including NaCl, HCl, $MgSO_4$, sucrose, and glumate [41, 42]. Based on the similar LAPS measurement setup, cell-to-cell communications between different types of cells have also been investigated to explore taste sensation and analyze taste-firing responses [43, 44]. LAPS chip has also been applied in the development of taste sensors for the detection of different bitter compounds based on extracellular recordings on taste receptor cells, which can respond to different bitter stimuli [37]. In addition, LAPS chip can realize the non-invasive measurement in real-time for a long term. The extracellular recordings of taste receptor cells can successfully discriminate different bitter stimuli such as $MgSO_4$, denatonium, and D-(-)-salicin. More recently, a new method for the label-free functional assays of olfactory and taste receptors have been developed based on localized extracellular acidification measurement with a LAPS chip, which have been successfully applied in the functional assays of a human taste receptor, hT2R4, and an olfactory receptor of *Caenorhabditis elegans* (*C. elegans*), ODR-10 [45]. This biosensor provides a valuable and promising approach for label-free functional assays of chemical receptors as well as for the research of other G protein-coupled receptors (GPCRs). In addition to LAPS, olfaction and taste sensors