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Introduction to Essentials of Machine Olfaction and Tastes

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Although there are a variety of sophisticated machines for visual and auditory senses, machines for chemical senses such as olfaction and taste are still prematured. However, they are very important since they are deeply related to our primitive but fundamental capabilities. We can search for foods in daily life as well as can avoid danger using olfactory sense. Although animal's capability is nowadays superior to human ones, we still have them. Those chemical senses cannot be ignored in our daily life. Nowadays we can create cyberspace made up of visual and auditory senses. However, that cyberspace still lacks reality since olfactory and gustatory senses are not included.

The first machine olfaction was proposed about 30 years ago. Then, it was extended and an electronic nose community appeared. Although many papers have been already published, its application toward to industry is still limited. Its sensitivity, selectivity, and robustness against disturbance should be much improved for the actual application. A variety of applications are waiting for its progress. This book describes the current effort of sensing part of machine olfaction.

Machine olfaction has another part such as olfactory display. It works as an actuator in olfaction. An olfactory display is relatively new compared with odor sensing technology. Researchers in virtual reality have focused on the olfactory display to realize cyberspace with chemical sense. Although researcher population of olfactory display is still small, it gradually spreads into the world.

A human olfactory interface has both odor sensing and olfactory display. It is now growing up in the field of human interface. Utilizing those two techniques, odor recorder and teleolfaction system are being studied.

In contrast to olfaction, a taste sensor has been applied to a certain application area. Especially, medical field is its good customer. However, we still wait for simple easy-to-use taste sensor to include taste sense in cyberspace. The attempt to realize it will be later shown.

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Recently we often hear the world such as cyber-physical system. Cyber-physical system enables cyberspace with physical senses. However, we have never heard the word "cyber-chemical system." We can have cyber-chemical system if the technologies of machine olfaction and taste are easily available.

This book describes the essential parts of machine olfaction and taste. Chapter 2 describes olfactory mechanism of a living body. Utilizing it, olfactory biosensor is being developed. Chapter 2 also explains the olfactory biosensor.

Chapter 3 shows odor sensing technology. It explains the basics of artificial sensors. Moreover, a large-scale sensor array in the same way as biological one is being studied. This trend in electronic nose is introduced in Chapter 3.

Chapter 4 shows the taste sensor. It describes the principle and its application toward foods and medicines. This chapter explains the latest research review as well as the fundamentals of taste sensor.

Chapter 5 describes the current pattern recognition technologies available in electronic noses. The pattern of many ORN responses is recognized by an olfactory neuron system. Thus, the output pattern of the array of sensors with partially overlapping specificities is recognized in machine olfaction. Chapter 5 describes the basics of pattern recognition technologies together with its advanced technologies.

Chapter 6 explains mobile robot technology with chemical senses. It can search for the target chemical in the field. Its sensor, algorithm to look for the target and the experiment is shown in this chapter.

Chapter 7 shows olfactory display and odor recorder. Various types of olfactory displays are systematically described. Moreover, the review of odor recorder is shown in this chapter.

Chapter 8 is the summary and describes the perspective of machine olfaction and taste.

Each chapter covers an essential part of machine olfaction and taste. It describes basic part at first and then extends their contents to the advanced technology.

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Physiology of Chemical Sense and its Biosensor Application

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2.1 Introduction

Odorant sensors for detecting various types of odorants are currently required increasingly for several applications, such as disease diagnosis, food administration, and risk management associated with detection of explosives and drugs. Odorant sensors based on metal-ode semiconductor devices, quartz crystal microbalances (QCM), or surface acoustic wave (SAW) detectors have been developed for a variety of odorant-detection applications. However, the performance of these sensors is still inferior to the olfactory systems of living organisms in terms of selectivity, sensitivity, and response time.

In animals olfaction plays a key role in the release of appropriate behavior under complexly changing environment. Animals extract adequate information from numerous odorants in their surroundings and respond in many aspects of the animal's life including foraging, prey detection, finding hosts, and mating. Odor information is detected by olfactory receptor neurons (ORNs) in an olfactory organ and properly processed in neural networks in the brain and finally translated into the appropriate behavioral responses, mechanisms of which are critically important in the development of advanced odor sensors and odor tracking robots based on biological systems as well as neuroscience and neuroethology.

Transduction mechanisms in olfaction have been revealed in vertebrates and insects. Odorants are detected at the surface of the olfactory epithelium which contains ORNs in vertebrates. Insects have ORNs in the sensillum of antennae. Odorant signals detected at the

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membrane of the ORNs are converted into electric signals and transmitted to the brain. In vertebrates the transduction is mediated by complex signal transduction pathways through G proteins, adenylyl cyclase, cyclic adenosine monophosphate, and cyclic nucleotide-gated ion channels (i.e., G protein-coupled receptors (GPCRs)). By contrast, insect odorant receptors, coupled with an olfactory receptor coreceptor (Orco), form ligand-gated ion channels (i.e., ionotropic receptors) that control all-in-one odorant reception and ion influx. Moreover, the insect ionotropic receptors can selectively detect various types of odorants covering a wide range of chemical functional groups, including alcohols, aldehydes, ketones, acids, hydrocarbons, and aromatic compounds.

Since the transduction mechanisms in animals have been revealed, odorant receptors would be valuable odorant sensors with high selectivity, high sensitivity, and good response time compared to conventional sensors. So far a number of cell-based odorant sensors have been studied and proposed by using recent advanced gene engineering techniques. Among those cell-based sensors, insects are equipped with sophisticated molecular mechanisms that involve initial activation of odorant receptors. The insect odorant receptors would be valuable odorant sensors with high selectivity, high sensitivity, and good response time and could be assembled into a compact chip to develop portable odorant sensors.

In this chapter, first, transduction mechanisms of insect and vertebrate ORNs are introduced. In addition transduction mechanisms of insect and vertebrate gustatory signals are also introduced. Based on these findings, various kinds of biological components such as tissues, sensory neurons, proteins, and genes regarding olfaction in living organisms have been utilized for application to olfactory sensors. Different types of olfactory sensors, that is, tissue-based sensors, cell-based sensors, and receptor-based sensors, are then introduced.

2.2 Olfaction and Taste of Insects

2.2.1 Olfaction

2.2.1.1 Anatomy of Olfaction

Structure of Olfactory Sensillum

Insects detect odorants with a pair of antennae on their head and, in some dipteran species, a pair of maxillary palp extending from the base of the maxilla (Figure 2.1a). ORNs are housed in cuticular specialization, named olfactory sensillum, on these olfactory organs (Figure 2.1b, c). Olfactory sensillum has numerous minute pores (10–100 nm), named olfactory pores [57, 128], which allow odorant molecules to enter inside the sensillum. ORNs are bipolar neurons that extend their dendrites, the site of odorant reception, into the sensillum and project their axons into the antennal lobe (AL), the first olfactory center of the brain in insects [41]. Cell bodies and inner dendrites of ORNs are surrounded by three accessory cells: the tormogen, trichogen, and thecogen cells (Figure 2.1c). These cells isolate a lymph space surrounding the outer dendrites of the ORNs from the hemolymph. Differences in chemical composition of the sensillum lymph and the hemolymph generate a standing electrical potential difference, the transepithelial potential (see Refs. [58, 98]). Odorant stimulation generates a receptor potential in the outer dendritic membrane, which can induce the generation of action potentials in a more proximally located spike-generating zone. Olfactory sensilla are classified to several types according to their outer shape (s. trichodea, s. basiconica, s. coeloconica, s. placodea,

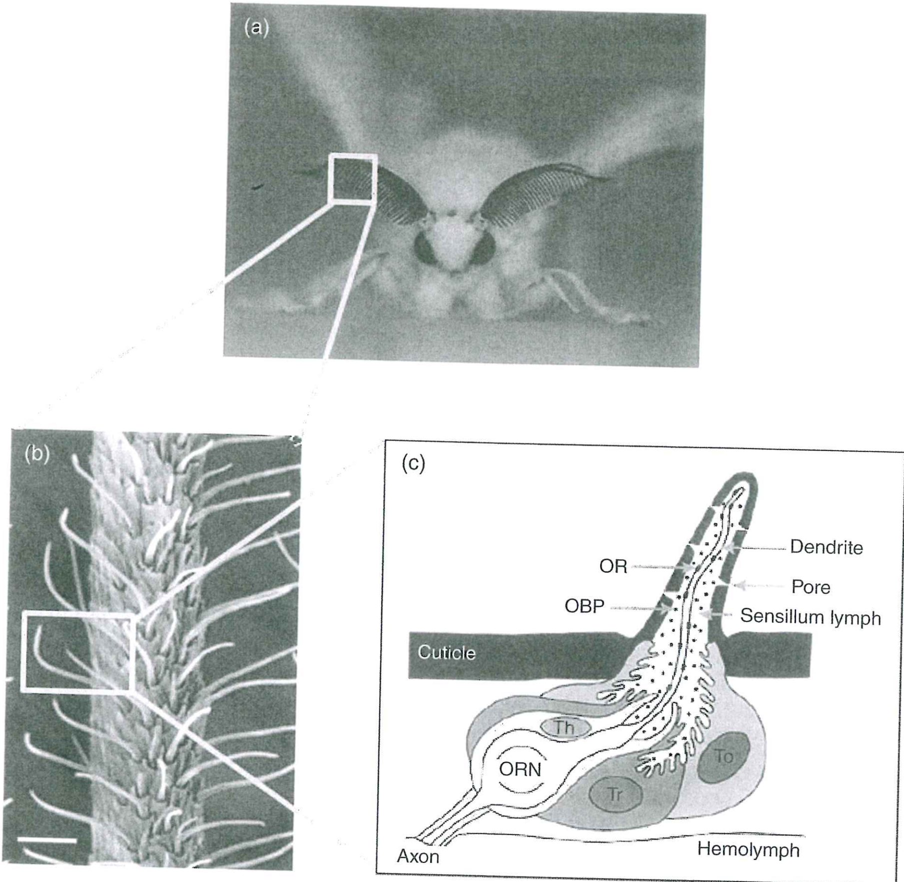


Figure 2.1 Main olfactory sensory organs of the silkmoth *Bombyx mori*. (a) A male silkmoth with its prominent antennae optimized for odorant detection. (b) Scanning electron micrograph of an antenna. Scale bar: 25 μm. (c) Schematic diagram of an olfactory sensillum. ORNs are surrounded by three types of accessory cell: tormogen (To), trichogen (Tr), and thecogen cells (Th). (Figure (c) is reproduced with permission from Ref. [48]. © Springer.)

and so on). In some cases, the type of sensillum is well correlated with its function. For example, s. trichodea in male moths house ORNs specifically tuned to conspecific sex pheromones [52, 53], while others house ORNs for so-called general odorants such as from foods or plants [108].

Detection of Odorants by ORNs

Odorant molecules in the air are first absorbed on the cuticular surface of the sensillum, and then they diffuse inside the sensillum through olfactory pores and pore tubules [52, 54, 55]. Since most volatile odorants are hydrophobic in nature, it is difficult to efficiently pass

sensillum lymph to dendritic membrane of ORNs. Therefore, mechanism to facilitate solubilization of odorants into aqueous lymph layer is important to achieve sensitive detection of odorants. For this, insects utilize small (about 15 kDa) soluble globular proteins named odorant binding protein (OBPs) [141] that bind odorants and transport them to dendritic membrane of ORNs. Mechanisms of odorant binding and release by OBPs are well studied using the silkmoth (*Bombyx mori*) pheromone-binding protein 1 (BmPBP1) that binds sex pheromone components of that species. BmPBP1 has two different conformations that reversibly change in a pH-dependent manner [149]. At neutral pH, odorant binding pocket located inside of proteins is open for binding odorant, while at acidic pH C-terminal loop domain of PBP occupies this binding pocket [42, 73]. This conformational transition is believed to occur around dendritic membrane due to lower pH around cellular membrane, resulting in the release of odorant from internal binding pocket around ORs. Then, odorants are detected by OR complex that activate chemoelectrical transduction machinery on dendritic membrane of ORNs.

2.2.1.2 Signal Transduction of Odor Signals

Upon binding to OR, the information of odorants is converted into electrical signals in ORNs. Earlier studies have reported rapid and transient increase of G protein-mediated second messenger, inositol triphosphate (IP_3), in the antennal homogenates after pheromone stimulation [11]. Expression of heterotrimeric G protein in ORNs and activity of its effector enzyme in antennal homogenate were also demonstrated, suggesting that odorant signals are transduced into electrical signals via heterotrimeric G protein-mediated second messenger cascade (Figure 2.2a) [48, 67]. However, recent physiological analysis of ORs revealed that insect ORs form heteromeric complex with their coreceptor Orco (originally named as Or83b in *Drosophila melanogaster*) and function as an odorant-gated ion channel (Figure 2.2b, c) [121, 126, 147]. Orco is originally isolated as a member of insect ORs and has the following unique characteristics [68, 100, 144]: (i) Orco is exceptionally conserved across insect species, while conventional ORs are highly divergent within and across species. (ii) Orco is expressed in most ORNs, while conventional ORs are expressed in specific subsets of ORNs.

Sato *et al.* coexpressed BmOR1 with BmOrco and other combinations of members of the Orco family with ORs in heterologous expression systems. Examination of the electrophysiological properties of an Orco/OR complex revealed that it acts as a pheromone/odorant-gated nonselective cation channel (Figure 2.2b) [121]. Interestingly, there was no evidence for an elevation of second messenger levels upon stimulation with ligands, indicating no involvement of a G protein-mediated cascade in the activation of Orco/OR complexes. Later pharmacological analysis of cultured cells coexpressing Orco and ORs from *D. melanogaster* supported this conclusion [126]. In the meanwhile, Wicher *et al.* found that fast transient and slow prolonged ion currents occur in cultured cells coexpressing DmOrco and *D. melanogaster* ORs upon stimulation with appropriate ligands for the expressed ORs (Figure 2.2c) [147]. They proposed that fast currents result from direct activation of Orco by ORs and slow currents occur via G protein-mediated activation of Orco. Both studies indicated that odorant signals are mediated by odorant-induced channel activity of ORs/Orco complex or Orco but different in terms of involvement of G protein-coupled pathway. Further studies will be required to reach consensus about the roles of G protein-mediated second messenger system on reception of odorants.

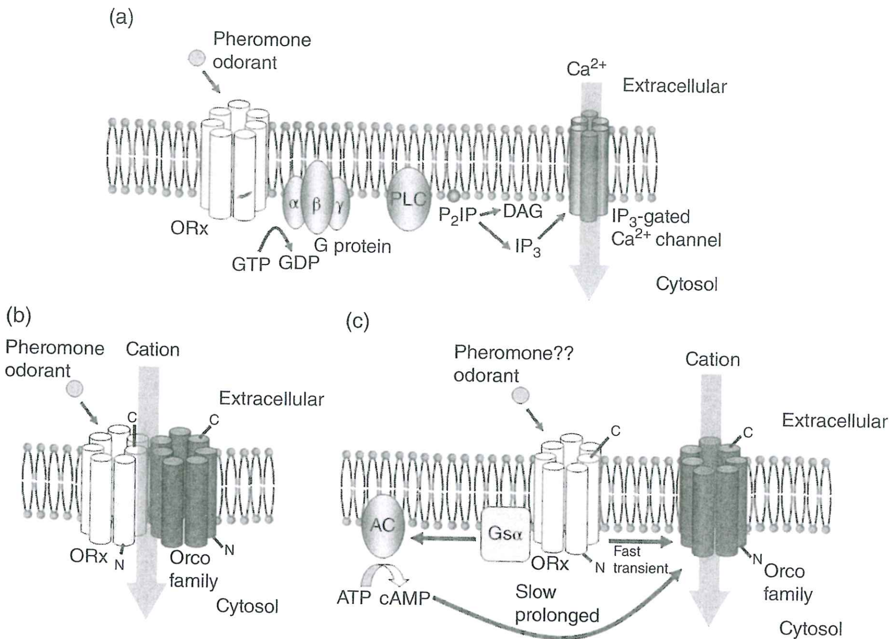


Figure 2.2 Proposed signal transduction mechanisms. (a) A classical model of insect olfactory transduction that involves a G protein-mediated PLC–IP_3 pathway. (b) Alternative model where the odorant receptor (OR) forms a heteromeric odorant-gated nonselective cation channel with an Orco family protein. (c) Alternative model that postulates two pathways. An ionotropic pathway involves the direct activation of Orco by an OR resulting in a rapid but transient cation influx. A metabotropic pathway is coupled to the G protein and induces slow but prolonged cation currents. (Reproduced with permission from Ref. [117]. © 2014, Sakurai, Namiki and Kanzaki.)

In this regard, phosphorylation of ORs by protein kinase C activated by presumably G protein-mediated second messengers reportedly enhances responses to odorants [36, 148], suggesting that second messenger system may not directly activate but modulate activity of Or/Orco channel through phosphorylation of ORs. More recently, it was reported that latency of electrophysiological responses of antennae of several insect species is as fast as several millisecond order [132]. This response speed is in accordance with the range of ionotropic pathway, indicating at least fast response is mediated by ionotropic activity of OR and Orco complex.

2.2.1.3 Molecular Biology of Olfaction

The insect OR gene family was first identified from the fruit fly by bioinformatics-based methods as well as large-scale screenings of olfactory tissue-specific genes [25, 34, 143]. Sixty OR genes are found in whole genome sequence of the fruit fly [144]. After that OR genes have been identified from various insect species. The number of OR genes considerably

varied between species ranging from 10 in the body louse to more than 300 in ants. Amino acid sequence comparison revealed that insect ORs form a unique gene family with no obvious homology with any other proteins including ORs from vertebrates. Although insect ORs possess seven-transmembrane domain characteristic to GPCR family, they have a reverse membrane topology compared to GPCRs with their N-terminal on the cytoplasmic side and C-terminal on the extracellular side [7, 49, 72, 89]. Indeed, recent physiological studies demonstrated that insect ORs form odorant-gated ion channel with Orco (see Section 2.2.1.2 in detail).

Response Profiles of ORs

In an OR and Orco channel complex, OR is responsible for ligand binding and determines response profiles of ORNs [121]. By now, response spectrum of more than 100 ORs has been determined by using "empty neuron" expression system in the fly antennae and/or heterologous cell expression systems such as *Xenopus* oocytes. In principle, each OR can bind different odorants and each odorant can be recognized by multiple ORs. Response spectrum of individual OR continuously distributed from narrowly to broadly tuned one [17, 38, 145]. Comprehensive analysis using the fruit fly has uncovered the relationship between ORs and ORNs as well as ORNs and glomerulus in the AL [28, 33]. Similar to vertebrate olfactory systems, most ORNs selectively express one of many ORs, and ORNs expressing the same OR project into a single defined glomerulus in the AL. Since each OR normally responds to various odorants and each odorant is detected by various ORs, odorant information is represented as a combination of activated glomeruli in the AL.

2.2.2 *Taste*

In the gustatory system, the sense of taste is essential for the animals to evaluate which food is good to eat and which food should be avoided. Compared to most mammals that can discriminate five basic tastes (see Section 2.3.2.1), insect basic tastes are divided into four categories: bitter, sweet (sugar), salty, and water. In addition to these tastants, insect gustatory system can detect uncanonical taste substances such as fatty acids, sour tastes, and chemicals unrelated to food such as contact pheromones. In this section, the mechanisms of taste detection in insects are briefly described.

2.2.2.1 Anatomy of Taste

Taste Organ

One of the striking features of the insect gustatory system is that taste organs are not restricted to mouth part but are distributed in multiple body parts. For example, in adult fruit flies, four appendages—the proboscis, legs, anterior wing margins, and ovipositor—possess gustatory function (Figure 2.3a) [79, 129, 142]. The proboscis is a long appendage extending from the head and comprises external taste organ named labella that is located at the apical end of the proboscis and three internal organs—the labral sense organs (LSOs), the dorsal cibarial sense organ (DCSO), and the ventral cibarial sense organ (VCSO) that are located along the pharynx (Figure 2.3b) [129]. These organs play roles in determining whether to ingest or expel food and thus can be regarded as the functional equivalent to the mammalian tongue. Taste organs

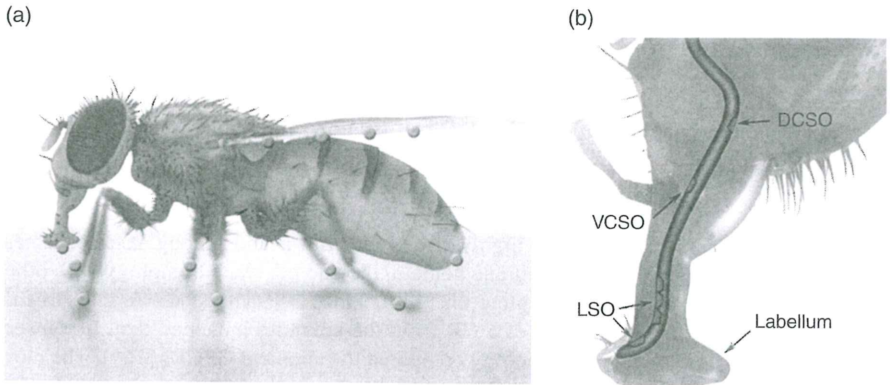


Figure 2.3 Taste organs of adult fruit fly, *D. melanogaster*. (a) Distribution of taste organs on adult fly body. (b) Taste organs in mouth part. (Reproduced with permission from Ref. [79]. © Elsevier.)

are present on distal segment of legs, tarsi. Taste sensors on tarsi carry out initial sampling of potential food and evaluate the quality of it. Tarsal taste organ on male forelegs also plays a role in detecting contact pheromones that promote or inhibit courtship behavior of males [10]. Taste organs on wings are indicated to participate in the detection of microbe-derived lipopolysaccharides that induce glooming behavior to remove microbe from fly's cuticle [157]. The ovipositor taste organ provides information of nutrient conditions to identify location suitable for egg laying [159].

Structure of Taste Sensillum and Gustatory Receptor Neurons

Taste substances (tastants) are detected by sensory neurons referred to as gustatory receptor neurons (GRNs) housed in taste sensillum on those organs. GRNs are bipolar neurons that extend their dendrite into the shaft of taste sensillum and project their axon to the suboesophageal ganglion, taste center in insect brain. In contrast to olfactory sensillum that has many pores on its cuticle, taste sensillum has a single pore at the apical end of the sensillum from which tastants enter into taste sensillum. Typically, there are one to four GRNs and one mechanosensory neuron in individual sensillum. Each GRN is tuned to substances of one of four basic taste categories.

2.2.2.2 Molecular Biology and Signal Transduction of Taste

Recent studies have revealed that tastants are detected by various types of receptors expressed in GRNs. Basically, the types of receptor correspond to taste categories. In this section, the types of receptors and signal transduction activated by interaction of tastants with receptors are summarized.

Bitter and Sweet (Sugar) Taste

Bitter and sweet tastes are detected by the large receptor family named gustatory receptor (GR) that is the major class of insect taste receptor. GR was first discovered from the fruit

fly by bioinformatics approach of nearly completed *Drosophila* genome sequences to seek candidate genes that can encode seven-transmembrane domain receptor [26, 123]. By these analyses, 43 GRs that belong to the novel membrane protein family and expressed selectively in subsets of GRNs were reported. Later analysis revealed that there are 68 GRs in whole genome sequences of *Drosophila*[114]. By now, GR family genes are reported from various insect species of different orders such as mosquito, moth, beetle, wasp, bee, aphid, and louse. Number of GR genes is different between species from 10 in honeybee *Apis melifera* to 114 in disease vector mosquito *Aedes aegypti*. Similar to insect ORs, amino acid sequences of GRs are highly divergent within and among insect species. Although GRs have seven-transmembrane domain, they do not share homology with other known GPCRs and membrane receptors and form independent receptor family. In this sense, membrane topology analysis of a GR from the silkmoth indicated that, like insect ORs, GR has inverted membrane topology compared to canonical GPCRs [164]. The closest relative of GR family is the insect OR family. Phylogenetic analysis revealed that the emergence of the GR family precedes that of the OR family, suggesting that ORs may evolve from GRs [114, 140].

GRs basically function as bitter and sweet taste receptors. Members of the GR family function as receptors for other categories such as an amino acid (L-canavanine) [29], nonvolatile contact pheromones [10], and CO_2[130]. Surprisingly, it is reported that member of GR family is also involved in the detection of nonchemical signals including light [155] and temperature [91], indicating highly divergent roles of GRs.

In many cases multiple GRs are expressed in individual GRNs, suggesting that GRs form heteromeric complex to exert their functions. In the meanwhile, at least 2 GRs tuned to fructose can be functionally reconstructed in heterologous expression system [122]. Thus, mode of action of GRs is still largely unknown.

Signal transduction pathway following activation of GRs is also a major open question in the insect gustatory system. Recent studies raise the possibility that GR signaling is meditated by both G protein-coupled metabotropic pathway and ionotropic activity of GRs. Regarding metabotropic pathway, the expression of heteromeric G protein in GRNs has been shown [134], and mutation or knockdown of effector gene of G proteins reduced physiological and/or behavioral responses to bitter and sweet tastants [29, 50, 60, 136]. The involvement of G protein-coupled pathway is also evident for tastants in other categories but mediated by GRs, including CO_2[160] and an amino acid (L-canavanine) [29]. Regarding ionotropic activity of GRs, at least one GR for fructose appears to function as a tastant-gated nonselective cation channel, independent of a G protein-coupled pathway [122].

Salty Taste

Insects equip two types of salt GRNs: one tuned to high salt and the other to low salt. In *Drosophila* larvae, two epithelial Na channel (ENaC) family members, PPK11 and PPK19, are required for response to low salt [81], whereas in adult flies member of IR family IR76b is required for low-salt detection [165], which encodes continuously open-state Na^+ leak channel. Because Na^+ concentration in sensillum lymph is much lower than that of the hemolymph, influx of Na^+ through ENaC and IR76b occurs when insects take food containing low salt. This influx depolarizes the GRNs. Identification of receptors responsible for detection of high salt is a major question of salt detection in insects.

Sour Taste

Unlike mammals, insects do not possess sour taste cells. Instead, it was shown that subsets of bitter GRNs mediate carboxylic acid signals [21]. In addition, the activity of sweet GRNs is inhibited by acids [21]. However, receptors for these acids have not molecularly identified yet.

Water Taste

GRNs that respond to low osmolality are used for detecting water. A member of the degenerin/epithelial Na channel (DEG/ENaC) family, named PPK28, responds to low osmolality and is necessary and sufficient for water sensitivity [15, 23].

2.3 Olfaction and Taste of Vertebrate

2.3.1 Olfaction

2.3.1.1 Anatomy of Olfaction

Terrestrial vertebrates utilize two kinds of olfactory information, volatile odorants and pheromones.

A main olfactory system, which is responsible for volatile odorant detection, consists of ORNs and the olfactory bulb (Figure 2.4a). ORNs are located in the olfactory epithelium lining the nasal cavity and are surrounded by glia-like supporting cells. There are also basal

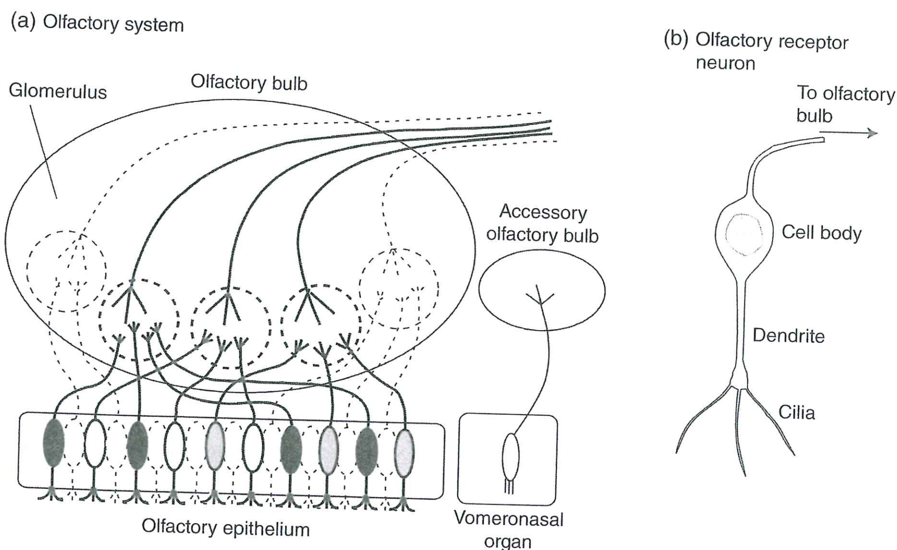


Figure 2.4 (a) Schematic diagram of vertebrate olfactory system. Each ORN in the main olfactory epithelium expresses only one odorant receptor gene and sends its axon terminal into one of the glomeruli in the olfactory bulb. ORNs expressing a given type of odorant receptor converge to a common glomerulus. Vomeronasal sensory neurons in vomeronasal organ project their axon into the accessory olfactory bulb. (b) Schematic drawing of an ORN. Odorant receptors and signal transduction machineries are expressed in the olfactory cilia

cells in the epithelium, which are kinds of progenitor cells that differentiate into ORNs or supporting cells. The olfactory epithelium has been divided into four zones based on odorant receptor expression. A specific type of ORN is located in one of the four zones [111, 137].

Each ORN extends a single dendrite to the surface of olfactory epithelium, and the dendritic knob projects 5–20 fine cilia in the mucus layer where odorant molecules can dissolve and bind to. The ORN extends an axon from the other end of the cell body and projects directly to the olfactory bulb in the forebrain where it synapses on the distal primary dendrites of second-order neurons, mitral cells, and tufted cells (Figure 2.4b).

As detailed in Section 2.3.1.3, ORNs express one of about 1000 odorant receptor proteins in the mouse. Although ORNs that express a particular odorant receptor distributed broadly in a given zone of olfactory epithelium, the axons from particular type of ORNs project to common glomeruli in the olfactory bulb (Figure 2.4a). This means that individual glomerulus represents a single type of receptor [97]. There are 5–10 million ORNs in the olfactory epithelium, and their axons converged to about 2000 glomeruli in the olfactory bulb. It has been estimated that several thousand ORN axons synapse onto the dendrites of only 5–25 mitral cells in each glomerulus [32], indicating that a considerable amount of convergence of olfactory information occurs in the glomeruli.

The vomeronasal organ (VNO), which is responsible for mediating pheromone information, is located at the base of the nasal septum in the mouse. Vomeronasal sensory neurons (VSNs) project axons via vomeronasal nerve to the accessory olfactory bulb, which is located on the dorsal posterior part of the main olfactory bulb (Figure 2.4a).

2.3.1.2 Transduction of Odor Signals

Signal Transduction Cascade in ORNs

The ORN depolarizes in response to an application of volatile odorants to olfactory cilia, which triggers action potentials to transmit the olfactory information along the olfactory receptor axon to the glomeruli in the olfactory bulb. Signal transduction is the process by which chemical information is transformed into an electrical signal within a cell. The signal transduction mechanism of ORN has been extensively studied in the past decades.

Figure 2.5 shows a schematic drawing of the signal transduction cascade of ORNs. A signal transduction takes place within the olfactory cilia. It initiates when volatile odorant molecules bind to an odorant receptor. Odorant-bound receptor activates ORN-specific GTP-binding protein, G_{olf}, which then activates adenylyl cyclase. As a result, second messenger cAMPs are produced from ATP by enzymatic activity of adenylyl cyclase and diffuse within cilia. cAMP binds to and opens cyclic nucleotide-gated (CNG) channels to generate an inward current that causes a depolarization of the cell. CNG channels are non-selective cation channels first found in retinal photoreceptors, which are opened by direct binding of cyclic nucleotide (cAMP in the case of ORNs). In physiological conditions, Na^+ and Ca^{2+} enter the cell through the CNG channels to carry positive charge. The Ca^{2+} influx causes an opening of calcium-gated chloride channels, resulting in an inward current (Cl^- efflux) that causes a depolarization.

It is interesting that both cationic current through CNG channels and anionic current through calcium-gated chloride channels are responsible for odor response, unlike, for example, phototransduction. The chloride component is reported to be as large as the cationic component [71].

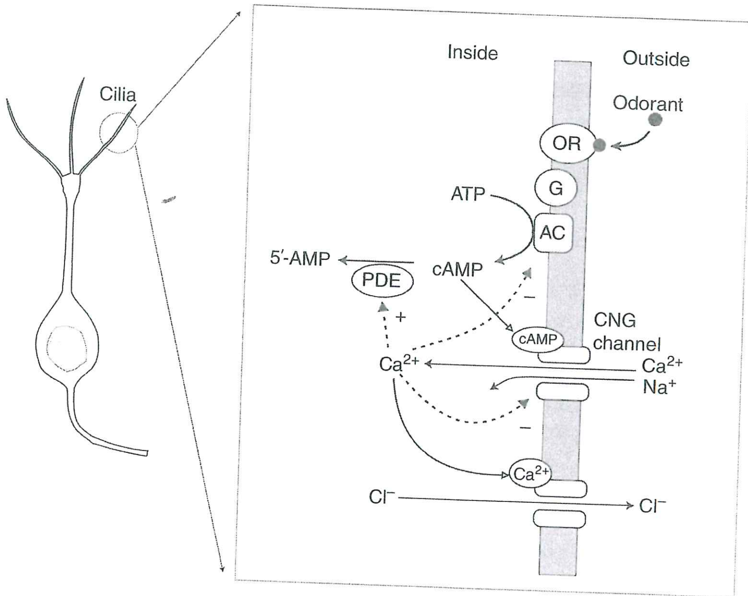


Figure 2.5 Schematic diagram of signal transduction cascade in ORNs. AC, adenylyl cyclase; CNG channel, cyclic nucleotide-gated channel; G, G protein; OR, odorant receptor; PDE, phosphodiesterase. Dashed lines indicate the effects of Ca^{2+} on AC, CNG channel, and PDE. +, facilitation; –, inhibition

Since the olfactory epithelium is exposed to the external environment, that is, freshwater, the cation concentration in the mucus may not be consistent. Therefore, it is important that the inward anionic current would compensate a reduction of cationic current due to a reduction of cation concentration [71].

Odor Adaptation

Many studies have shown that ORNs quickly adapt to odor stimulation. When the prolonged odor stimulus is applied, the depolarization in response to odor stimulation decreases with time although the odor stimulation still exists [70]. Another manifestation of odor adaptation has been demonstrated by double-pulse experiments. When a pair of brief, identical odorant pulses are applied to the cell, the response amplitude induced by the second pulse is remarkably small in comparison with the first response, if the interval between the pulses are sufficiently short. It recovers as the interpulse interval increases (Figure 2.6a) [69, 70].

The dose–response relationship of odor responses shows that the dynamic response range under control conditions is extremely narrow. On the other hand, under the adapted states, the dynamic range of the odor responses shifts and broadens (Figure 2.6b) [69]. The adaptation is important for the ORN to work over a wide range of odorant concentration. When background odors are present, the sensitivity to odor stimulus decreases so that higher concentration of odors can be detected without saturating.

The next question is how the odor adaptation occurs. Kurahashi and Shibuya [70] reported that removal of external Ca^{2+} almost completely abolished the response decay during a

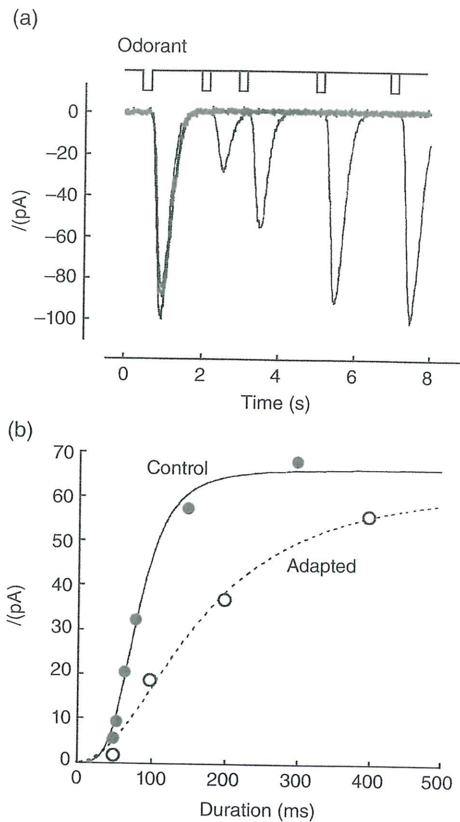


Figure 2.6 Odorant adaptation in an ORN. (a) Pulses of the odorant at the same concentration and duration were applied to the cell at different intervals. Response amplitude to the second pulse reduces depending on the intervals. (b) Relations between stimulus and response of an isolated ORN under control (filled circles) and adapted (open circles) conditions. (Reproduced with permission from Ref. [69]. © Macmillan Publishers Ltd.)

prolonged odorant stimulus, indicating that Ca^{2+} entry is responsible for odor adaptation. The molecular mechanism of odor adaptation mediated by Ca^{2+} has been studied since then.

It has been reported that odorant-induced increase in intracellular Ca^{2+} concentration reduces the cAMP sensitivity of CNG channel [66]. Two categories of adaptation mechanisms mediated by Ca^{2+} have been proposed. One is a direct action of Ca^{2+} on the CNG channel. Ca^{2+} entering through CNG channel binds to calmodulin which is the common calcium-binding protein, and the Ca^{2+}/calmodulin directly inhibits the channel itself [22]. As a result, the open probability of CNG channels decreases, causing a reduction of inward current, and the cell hyperpolarizes.

When ORNs are stimulated for a long period, the decay time course of response stimulated by odor is faster than that stimulated by the photolysis release of caged cAMP, indicating that

adaptation of odor response occurs upstream to adenylyl cyclase [133]. One candidate is type III adenylyl cyclase which is expressed in ORNs. Activity of adenylyl cyclase is inhibited by Ca^{2+}/calmodulin-dependent protein kinase and thereby reduction of cAMP production occurred [146]. Another candidate is phosphodiesterase which is a cAMP catabolic enzyme. Ca^{2+}/calmodulin-dependent phosphodiesterase (CAM-PDE) is expressed in ORNs, and CAM-PDE activity is elevated by Ca^{2+} stimulation. It is suggested that Ca^{2+} stimulation of CAM-PDE is necessary for odor adaptation [9, 156].

Signal Transduction Cascade in VSNs

VNO is responsible for detecting pheromones that are chemical substances produced and released by an animals and send information to other animals of mainly the same species. The pheromone molecules are received by receptor proteins expressed in the VSN. The signal transduction mechanism in VSNs is totally different from that in ORNs. Recent studies by molecular genetics have revealed that mouse VSNs express two distinct families of GPCRs, V1R [30] and V2R [40, 92, 116]. Evidences of downstream signal transduction mechanism in VSNs have also been reported. Transient receptor potential channel 2 (TRPC2) is exclusively expressed in VSNs, and the protein is highly localized to VSN sensory microvilli in which the sensory transduction is supposed to take place [78]. The TRPC2 is gated by the lipid messenger diacylglycerol (DAG) that is independent of Ca^{2+} or protein kinase C [88]. From the above evidences, the signal transduction scheme can be proposed as follows. The pheromone molecules are received by V1R or V2R, which then activate phospholipase C (PLC). DAG is synthesized from PIP2 by enzymatic activity of PLC and directly opens the TRPC2 to produce electrical signal. Recent study has shown that increase in intracellular Ca^{2+} concentration is caused by an opening of TRPC2 and such a Ca^{2+} increase regulates the opening of TRPC2 via Ca^{2+} calmodulin, which functions as negative feedback. This negative feedback may be an underlying mechanism of sensory adaptation in VSNs [127].

Recently, it is revealed that Ca^{2+}-activated chloride channels are involved in signaling in VSNs. Stimulus-induced opening of TRPC2 allows Ca^{2+} entering the cell. An increase in Ca^{2+} leads to opening of the Ca^{2+}-activated chloride channels, which amplifies the sensory responses in VSNs [61, 158].

2.3.1.3 Molecular Biology of Olfaction

Cloning of Odorant Receptor Gene

The odorant receptor gene has been cloned for the first time from rat olfactory epithelium using polymerase chain reaction by Buck and Axel [12]. They discovered a large gene family composed of about 1000 different genes, the largest gene family in mammals, which are responsible for the animal to recognize thousands of complex odors. The odorant receptor gene may account for about 2% of the genome. In humans, about 350 odorant receptor genes have been cloned. It has been widely believed that each ORN expresses only one of the odorant receptor genes, which is known as one receptor–one neuron rule [125].

Odorant receptor protein is a member of the GPCR family. Like other GPCRs, odorant receptor contains seven hydrophobic transmembrane domains (Figure 2.7). There is highly conserved pair of cysteines in the unusually long second extracellular loop, which are specific characteristics for odorant receptors. One of the most significant features of odorant receptors

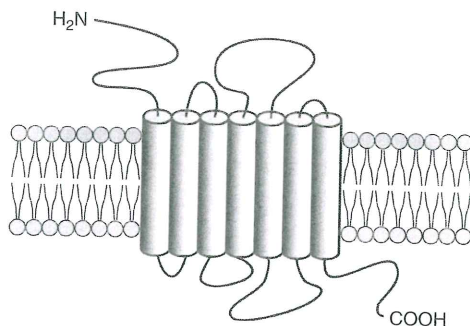


Figure 2.7 Characteristic structures of the odorant receptor. Each odorant receptor contains seven α-helical transmembrane domains, shown as cylinders

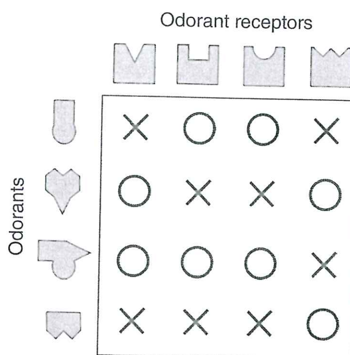


Figure 2.8 Combinatorial coding of odorants. A single odorant receptor recognizes multiple odorants, whereas a single odorant can activate multiple odorant receptors

is their large variability in amino acid sequence in the third, fourth, and fifth transmembrane domains. It is suggested that the binding of an odorant molecule occurs in an odorant binding pocket that is reportedly formed by the third, fifth, and sixth transmembrane domains. These features may account for the odorant receptors to recognize structurally diverse odorants.

Coding of Odor Information

It has long been known that humans can discriminate more than 10000 odors. Recent study estimated that humans can even discriminate at least one trillion olfactory stimuli [13]. There arises a question whether we can discriminate so many odorants with 350 odorant receptors. Although each ORN expresses single odorant gene, odor molecules are recognized by more than one ORN. The odorant receptors also recognize multiple odor molecules (Figure 2.8). For example, mOR-EG, a mouse odorant receptor that was isolated from a eugenol-responsive ORN, recognizes 22 odorants, whereas some other receptors recognize only a small number of odorants [56]. Physiological experiments showed that a single odorant elicited electrical response in multiple ORNs and the response amplitudes varied. The recognition of an odorant

depends on which receptors are activated and to what extent. It has been revealed that each odorant receptor recognizes a specific structural feature in individual odor molecules. Therefore, each odorant or odorant mixture is encoded by multiple odorant receptors (Figure 2.8). The combinatorial nature of the olfactory code underlies the reason how humans can discriminate a huge number of odors.

2.3.2 Taste

2.3.2.1 Anatomy of Taste

In the gustatory system, the sense of taste is essential for the animals to evaluate which food is good to eat and which food should be avoided. Humans and most of other mammals can discriminate five basic tastes—sweet, bitter, sour, salty, and umami—unlike the olfactory system that can discriminate thousands of odorants. Sweet, salty, and umami are appetitive tastes, and bitter and sour are aversive tastes. Foods with sweet, salty, and umami taste are usually required for energy, ionic balance, and synthesizing proteins, while bitter foods are very likely poisonous and sour foods are unripen or spoiled.

Tastants are detected by taste receptor cells in taste buds in which 50–150 taste receptor cells clustered. Taste buds are located on the tongue, and there are three types of structures called papillae (Figure 2.9a). Fungiform papillae that are located in anterior two-third of the tongue contain one or a few taste buds. Foliate papillae that are located in the posterior edge of the tongue and circumvallate papillae that are situated on the very back of the tongue both contain hundreds of taste buds. The taste bud is embedded in the epithelium. The taste pore is a small opening at the surface of the tongue where taste receptor cells are exposed to taste stimuli (Figure 2.9b). Each taste receptor cell is spindle shaped and extends its microvilli to the taste pore, allowing tastants to bind to the taste receptor proteins in microvilli (Figure 2.9c).

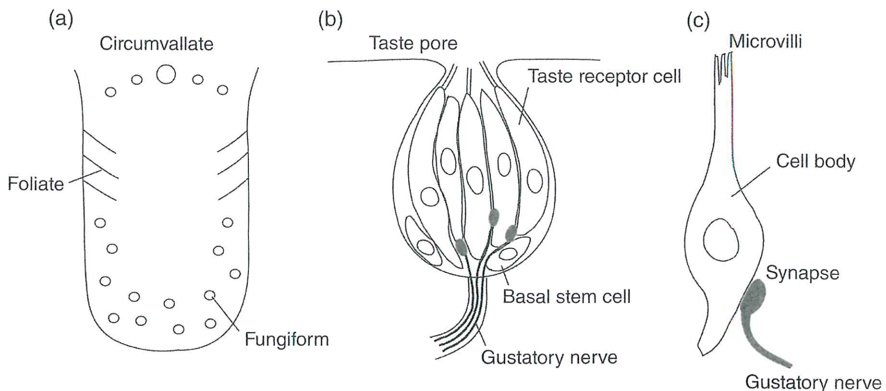


Figure 2.9 Schematic drawing of tongue, taste buds, and taste receptors. (a) There are three types of papillae: circumvallate papillae, foliate papillae, and fungiform papillae. (b) Each taste bud contains 50–150 taste receptor cells that extend their microvilli to the taste pore. (c) Structure of single taste receptor cell. Microvilli are located at the apical end of the cell

On binding tastants to the taste receptor protein, the cell depolarizes through signal transduction pathway. The taste signal is then transmitted to the gustatory nerve via synapse and transmitted to the brain.

2.3.2.2 Transduction of Taste Signals

Tastants are highly diverse in terms of their chemical structure. Salty and sour stimuli are simple ions such as Na^+ and H^+, while sweet, bitter, and umami substances are more complex, such as saccharides, alkaloids, and proteins. Therefore, the structures of receptor proteins and signal transduction mechanisms are varied among the five basic tastes.

Signal Transduction Cascade in Bitter, Sweet, and Umami Taste Receptor Cells
There have been many reports about taste transduction cascade in various vertebrate species, and the proposed hypotheses have wide diversity of signaling pathways (e.g., Kinnamon [62]). However, recent results have demonstrated that sweet, bitter, and umami taste receptors have a common signal transduction cascade (Figure 2.10a) [162].

The receptor proteins of sweet, bitter, and umami are GPCRs. The gustatory signaling starts with the binding of ligands to the receptor proteins followed by a conformational change in the receptor proteins. Activated receptors then activate a taste receptor cell-specific G protein

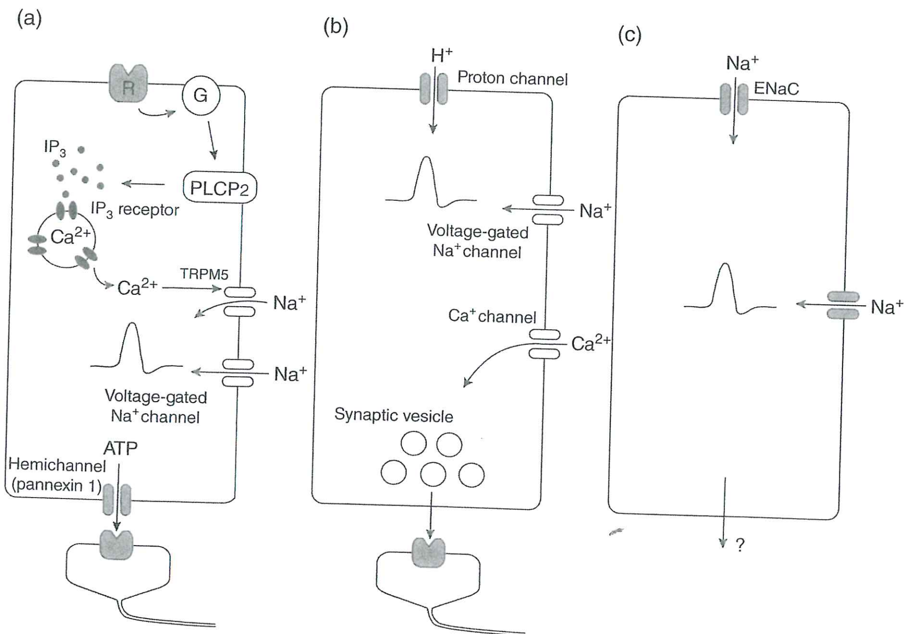


Figure 2.10 Schematic drawing of signal transduction cascades of sweet, bitter, umami (a), sour (b), and salty (c) tastes. ENaC, epithelial sodium channel; G, G protein; IP_3, inositol triphosphate; PLC, phospholipase C; R, receptor protein

gustducin that activates phospholipase C β_2 (PLC-β_2). Gustducin has high sequence homology to photoreceptor specific G protein transducin that is also expressed in taste buds [93]. IP_3 and DAG are generated from the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) by an enzymatic activity of PLC-β_2. IP_3, a water-soluble second messenger, diffused in the cytosol and binds to IP_3 receptor and causes an opening of the IP_3 receptor/channel leading to the release of Ca^{2+} from intracellular Ca^{2+} stores. The released Ca^{2+} gates TRPM5, a TRP channel family, in the plasma membrane [106, 163]. As a result, Na^+ enters the cell through TMPM5 channel to generate depolarization, which leads to an opening of hemichannel at the basal end of the cell. It has been reported that the taste receptor cells can elicit action potentials that are induced by TMPM5 channel-mediated depolarization. The recent study suggested that the action potentials may be required to open the hemichannels [99].

Signal Transduction Cascade in Sour and Salty Taste Receptor Cells

Tastants of sour and salty are simple ions, H^+ and Na^+, respectively. Therefore, the signal transduction cascades of sour and salty are relatively simple compared to those of sweet, bitter, and umami.

A number of candidates of sour receptors have been proposed over the years. For example, acid-sensing ion channels (ASICs) found in rats are cation channels that are activated by extracellular protons. However, they are unlikely to be a common sour receptor because they are not expressed in mouse taste buds [112]. Other candidates include hyperpolarization-activated cyclic nucleotide-gated channels (HCNs), K^+ channels, and TRP channel PKD2L1 (and/or PKD1L3), but there had been no direct evidences that those candidates are sour receptors [115].

More recently, Chang *et al.* [20] reported that responses of the PKD2L1-expressing taste cell to sour stimulus are mediated by a proton conductance and not mediated by Na^+-permeable channels as previously thought. In this model, protons enter into the sour cell through the proton channel at the apical end of the cell, which causes depolarization leading to a generation of action potentials. As a result, voltage-gated Ca^{2+} channels open, resulting in a rise in intracellular Ca^{2+} concentration at the basal end of the cell followed by a neuro-transmitter release (Figure 2.10b).

Another possible signal transduction pathway is mediated by intracellular acidification caused by membrane-permeable acids. It has been reported that several two-pore domain potassium (K_2P) leak channels are sensitive to acidification [113]. Blocking of K_2P channel by intracellular acidification would generate membrane depolarization, which enhances the depolarization caused by proton channels. This may explain why weak membrane-permeable acids taste sourer than strong acids, such as HCl.

The signal transduction mechanism of salty taste receptor has not been determined yet. A candidate of Na^+-permeable salty receptor is amiloride-sensitive epithelial Na^+ channel (ENaC). Upon application of Na salt, Na^+ passively enters the cell through the ion channel, generating membrane depolarization of the cell (Figure 2.10c).

Synaptic Transmission from Taste Receptor Cells to Second-Order Neurons

The sweet, bitter, and umami taste receptors have a unique mechanism for synaptic transmission from the receptors to second-order neurons. Unlike the conventional chemical synapses, those receptor cells do not express voltage-gated Ca^{2+} channels and synaptic vesicles. The recent studies showed that ATP is released by taste stimulation from type II taste receptor cell

that is thought to be sweet, bitter, or umami receptor, suggesting that ATP is a neurotransmitter of these cells. It is suggested that an unconventional depolarization-activated ATP release channel, most likely pannexin 1 hemichannel, was involved in the ATP release [99].

Ionotropic purinergic receptors P2X2 and P2X3 were first discovered in the afferent nerves that innervate taste buds by Bo *et al.* [8]. Double knockout of P2X2 and P2X3 eliminated taste responses in the taste nerves, and stimulation of taste buds *in vitro* evoked ATP, confirming that ATP is the neurotransmitter between the taste receptors and the taste nerves for sweet, bitter, and umami tastes [31].

Synaptic transmission from sour or salty taste cells to taste nerves is not clear yet. Responses to sour and salty stimuli were also abolished in P2X2/P2X3 double knockout mice, although ATP release has not been detected in type III cells that are thought to be sour or salty receptor cells. The mechanisms of synaptic transmission for these cells remain unclear.

2.3.2.3 Molecular Biology of Taste

Bitter, Sweet, and Umami Receptors (G Protein-Coupled Taste Receptors)

The taste receptors for bitter, sweet, and umami have been cloned recently and best understood among the five tastes. These receptors are taste-specific GPCRs which are expressed in each subset of taste receptor cells. There are two classes of GPCRs, T1Rs and T2Rs. Compared to other GPCRs like neurotransmitter receptors, the binding affinity of taste GPCR is generally low (in mM order), which is consistent with the physiological concentration of nutrients in foods.

In 2000, a novel family of GPCRs, T2R family, was first identified from genomic databases, and T2Rs are responsible for detecting bitter taste. T1Rs are A-type GPCRs that are similar to the opsins and the odorant receptors (Figure 2.11). Chandrashekar *et al.* [18] showed that specific T2Rs function as bitter taste receptors using a heterologous expression system. The T2R family comprises about 30 in mammals, and each taste receptor cell expresses a large repertoire of T2Rs, which can explain why taste receptors can detect many structurally diverse chemicals that are bitter to humans [1, 18]. It may be reasonable that the bitter taste receptor cells express most of T2Rs, which means that bitter taste receptor cells cannot distinguish bitter chemicals, because bitter sense evolved to avoid toxic substances. However, physiological study of bitter taste receptor cells showed that most of them were activated by only one out of five bitter chemicals tested, suggesting that bitter-sensitive taste receptor cells could discriminate bitter compounds [14].

Another class of GPCR was T1Rs that are responsible for detecting sweet and umami tastes. T1Rs are C-type GPCRs with large N-terminal domains (Figure 2.11). Three different subunits—T1R1, T1R2, and T1R3—have been identified [101]. T1Rs can only function as a heterodimer. The receptor with a combination of T1R2 and T1R3 can detect sweet substances [101], whereas a combination of T1R1 and T1R3 can detect most of the standard amino acids that cause umami taste (Figure 2.11) [102].

Sour and Salty Receptors

Although candidates for sour receptors including ASICs, HCN1 and HCN4 channels, K^+ channels, and the TRP channels PKD2L1 and PKD1L3 have been proposed, there have been almost no evidences for these candidates. More recently, Huang *et al.* [44] showed that

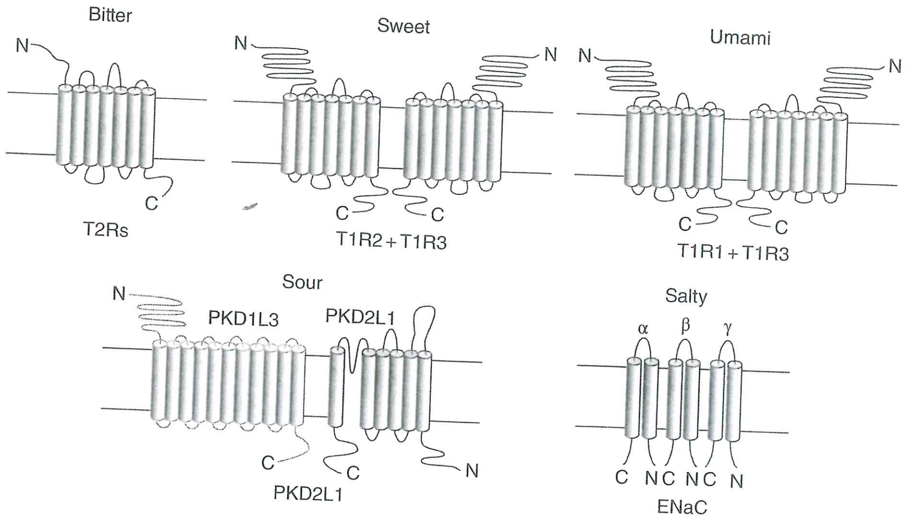


Figure 2.11 Characteristic structures of the taste receptors for five basic tastes. Bitter, sweet, and umami receptors are G protein-coupled receptors. Bitter receptors are T2Rs, while sweet and umami receptors are another class of GPCR, T1Rs. The heteromeric complex T1R2 + T1R3 recognizes sweet substances, while the complex T1R1 + T1R3 recognizes umami substances. Sour receptor PKD2L1 is coexpressed with PKD2L1

the mice lacking the taste receptor cell that expresses PKD2L1 did not respond to sour stimuli, indicating that PKD2L1 is serving as a sour receptor. It is also demonstrated that PKD1L3 is coexpressed with PKD2L1 in circumvallate and foliate taste receptor cells (Figure 2.11) [47].

Two types of salty taste, low and high concentrations of NaCl, have been proposed from behavioral responses. Low salt triggers attractive response, whereas high salt triggers aversive response. Since the attractive response is inhibited by amiloride that is a potent inhibitor of the epithelial sodium channel (ENaC), ENaC has been considered as a candidate for low-salt receptor. This channel is constituted of α-, β-, and γ-subunits (Figure 2.11) [16]. Recently, Chandrashekar *et al.* [19] showed that the genetically engineered mice with taste receptor cells lacking ENaCα completely eliminated salt attraction and sodium taste responses, indicating ENaCs function as low-salt receptors. The mechanism of high-salt reception is not known.

2.4 Cell-Based Sensors and Receptor-Based Sensors

Natural living organisms have equipped the sophisticated olfactory systems with their evolutions. The systems possess the capability to detect environmental odorants with higher performance than we expected. Recently, the mechanisms of the olfactory system have been gradually elucidated from long years of efforts by many researchers as mentioned in earlier sections. Based on these findings, we have become able to utilize various kinds of biological components, such as tissues, sensory neurons, proteins, and genes regarding olfaction in living organisms, for application to biosensors (Figures 2.12 and 2.13). Application of these biological

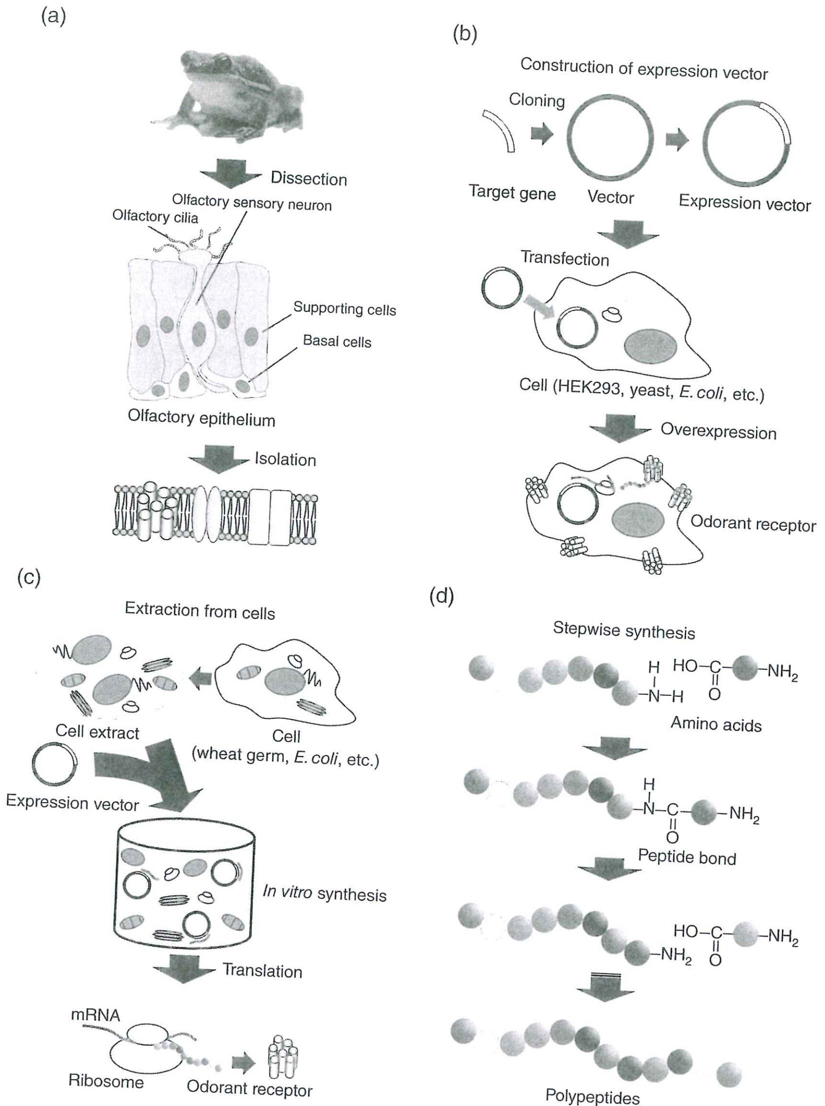


Figure 2.12 Schematic drawings of odorant receptor-production methods for cell-based or receptor-based sensors. (a) Isolation from living organs. (b) Cell-based production. (c) Cell-free production. (d) Chemical production

functions would lead to the development of odorant sensors with higher performances superior to the existing odor sensors in terms of sensitivity and selectivity. In this section, three types of biosensors, that is, tissue-based sensors, cell-based sensors, and receptor-based sensors, are introduced.

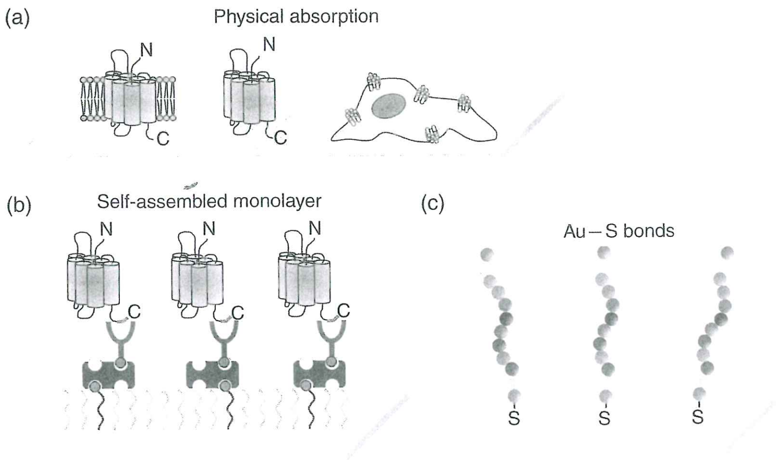


Figure 2.13 Schematic drawing of immobilization methods of odorant receptors, cells expressing odorant receptors, and polypeptides on the surface (gold) of transducers. (a) Physical absorption. (b) Self-assembled monolayer. (c) Au—S bonds

2.4.1 *Tissue-Based Sensors*

Surgically resected olfactory epithelium and mucosa tissue are directly applicable to electronic integrated devices for making biomimetic electric nose. Liu *et al.* showed that extracellular potential of rat's isolated olfactory epithelium and mucosa tissue could be detected *in vitro* by a microelectrode array (MEA) device and a light-addressable potentiometric sensor (LAPS) [83, 84]. As schematically shown in representative examples (Figures 2.14 and 2.15), in these systems, tissues contact with the conductive sensor device without injury, and thus the electrical signals from living olfactory cells in tissues could be measured. In contrast to needlelike electrodes, the noninvasive nature of MEA and LAPS for olfactory epithelium and mucosa tissue is due to their planar sensing station. They demonstrated that the biohybrid sensing systems could reflect characteristic firing patterns of olfactory epithelium to some volatile organic compounds (VOCs).

Organs of living body are also useful for sensitive detection of several volatile compounds (VCs). For instance, Park *et al.* utilized electroantennograms (EAGs) of some insects, vinegar fly, moth, and wasp (*D. melanogaster*, *Heliothis virescens*, *Helicoverpa zea*, *Ostrinia nubilalis*, and *Microplitis croceipes*) which possessed their own EAG response profiles to 20 different VCs [105]. They successfully recorded EAG responses to several VCs using antenna array consisting of four different insect's antennae mounted on the Quadro-probe EAG recording system (Figure 2.16). Rains *et al.* developed a portable device, Wasp Hound®, employing a trained living wasp (*M. croceipes*) as the sensor element [110]. The device is composed of a ventilated chamber as an insect cage equipped with a camera. It could quantitatively clarify the searching behavior of trained wasps and successfully detected the behavioral responses to the target odor.

Though higher organism's olfaction mechanism has still missing piece of the puzzle, vertebrates are very usable for easy odorant detection such as a typical case of sniffer dog. It's well known that canines have been used for detecting illicit drugs, several explosives, and human

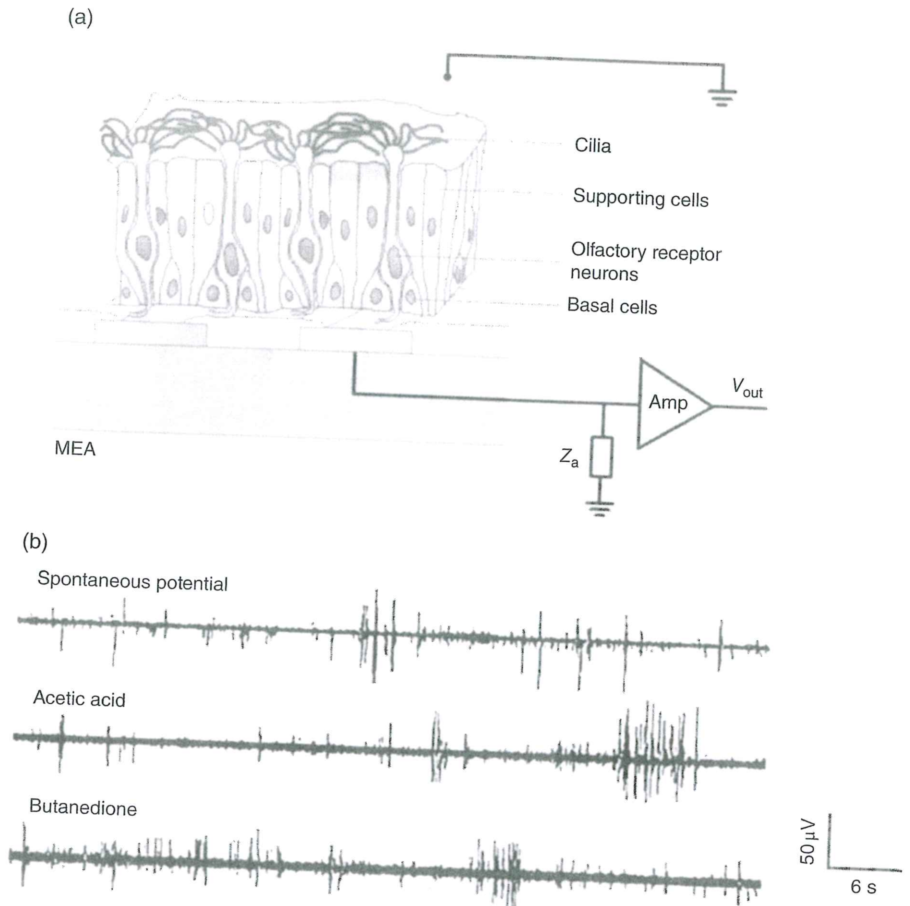


Figure 2.14 Study example of extracellular potentials recording by MEA. (a) Schematic view of olfactory receptor neurons in the epithelium on MEA. (b) Electrophysiological signals induced by the chemical substances. From Ref. [84]. Reproduced with permission from Elsevier

identification. Gazit and Terkel reported that canines could detect explosives with a probability of about 90% in actual outdoor space [35]. Similarly, rats have been applied to VC detection especially for exploring landmine and explosives from the 1970s onward [103, 107]. However, the fact remains that there are disadvantages in that the sensitivities are affected by their physical conditions and their trainings cost money and time.

As summarized in Table 2.1, rat was frequently used for tissue-based odorant sensors. The reasons for this trend may be came from the fact that rat is very easy experimental sample and an abundance of the anatomical insight. At the same time, utilization of some insects is also seen in late years.

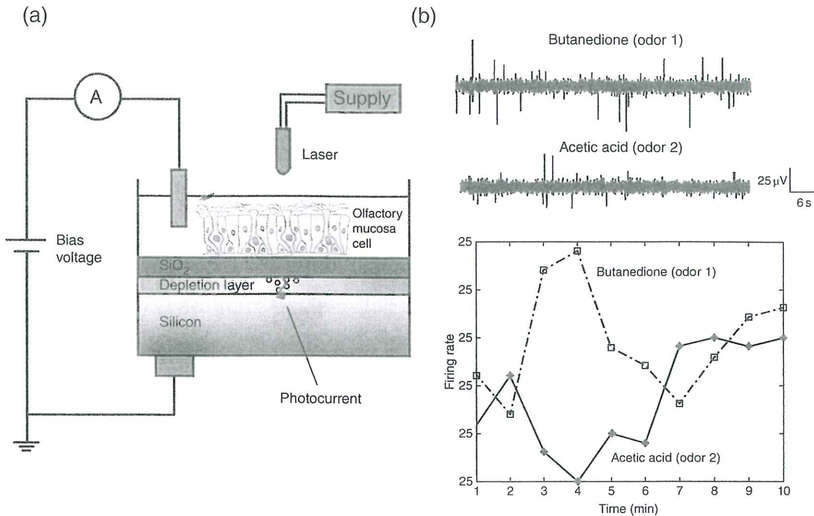


Figure 2.15 LAPS system applied to olfactory mucosa tissue cells. (a) Outline drawing of the LAPS system. (b) Firing patterns and the time courses evoked by chemical stimuli. From Ref. [85]. Reproduced with permission from Elsevier

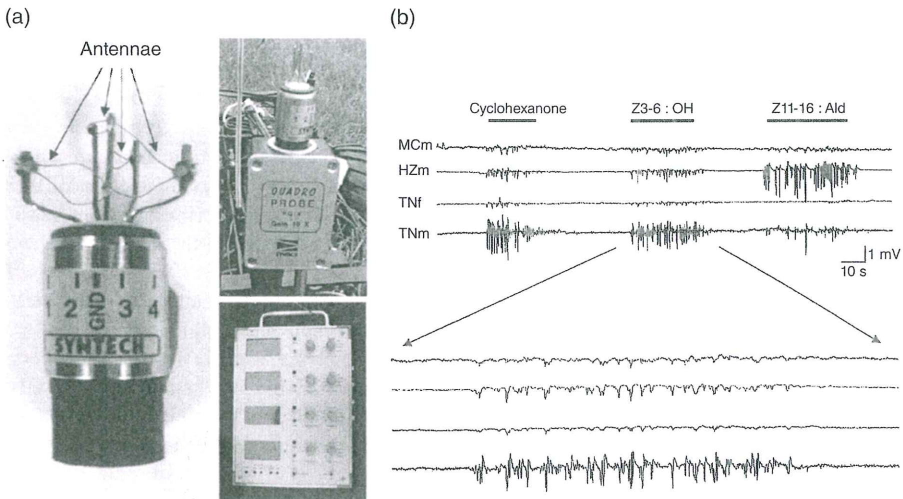


Figure 2.16 (a) Pictures of portable EAG recording system. (b) Simultaneously recorded four antennae EAG responses and the partial expansion patterns triggered by three different stimuli. From Ref. [105]. Reproduced with permission from Oxford University Press

Table 2.1 Summaries of tissue-based sensors

Tissue/organ/bion	Tested compound	Transducer	Tested concentration	Literature
Olfactory epithelium (Sprague-Dawley rat)	Butanedione, acetic acid	MEA	25 µmol/ml	[84]
	Ethyl ether, acetic acid, butanedione, acetone	MEA	10 µM	[86]
	Butanedione, acetic acid	LAPS	25 µmol/l	[83]
Olfactory bulb (Wistar rat)	Butanedione, acetic acid	LAPS	25 µmol/l	[85]
	Glutamic acid	MEA	10 µM to 5 mM	[24]
Antennae (insects: *D. melanogaster*, *Heliothis virescens*, *Helicoverpa zea*, *O. nubilalis*, *Microplitis croceipes*)	Z11-16:Ald, Z3-6:OH, hexanoic acid, benzyl acetate, 2-methyl-5-nitroaniline, cyclohexanone, α-pinene, *cis*-nerolidol, *trans*-nerolidol, β-caryophyllene, β-ocimene, (*R*)-(+)-limonene, methyl jasmonate, 2-diisopropylaminoethanol, indole, 2,2-thiodiethanol, 1-heptanol, 1-octanol, 1-nonanol, 1-decanol	EAG	10 or 100 µg/µl	[105]
Insect (*M. croceipes*, *H. virescens*, *H. zea*)	3-Octanone, myrcene	Behavior observation by camera	25–26 µmol/l	[110]
Canine (Belgian Malinois, Labrador Retriever)	C-4 explosive	—	30 g	[35]
Rat (*C. gambianus*; giant African pouched rat)	TNT and landmines	—	—	[107]

2.4.2 *Cell-Based Sensors*

As compared to tissue, isolated cell seemed to be more suitable for biosensing application due to easy isolation and transformation processes by developed bioengineering of recent years. Most studies of cell-based sensor were actively performed after entering the 2000s. Here, we mention some typical cultivated neurons and cells which have been utilized as cell-based odorant sensors. In either example, researchers used electrical, or resonant, or optical detection system as the transducer of chemical signal to acquire each output signal.

Insect's ORN and olfactory sensory neuron (OSN) which are in their antenna have been used for chemical biosensing applications. Huotari reported that the antenna of blowfly (*Calliphora vicina*) was specifically sensitive to 1,4-diaminobutane, hexanol-1, and butanoic acid [45]. In that study, *in vivo* action potentials from ORN in antenna of *C. vicina* were investigated by EAG, and the results showed that a higher amount of odorant substance caused saturation of the corresponding ORN response. Huotari and Lantto developed an analysis system for extracellular action potentials [46]. They applied the system for measurement of action potentials of *C. vicina*, mosquitoes (*Aedes communis*), pine weevils (*Hylobius abietis*), and trogossitid beetle (*Trogossita japonica*). They eventually revealed that the relationship

between action potential responses of ORNs and odor concentrations obeyed the power law. Liu *et al.* and Wu *et al.* used LAPS for monitoring of OSNs' extracellular potentials [82, 151]. The OSNs were cultivated on the surface of sensor chip, and the cells could be maintained for 1 week in LAPS device. Corcelli *et al.* studied OSNs' responses to two typical explosives, cyclotrimethylenetrinitramine (RDX) and trinitrotoluene (TNT) [27]. Electro-olfactogram recording and calcium imaging of rat olfactory mucosa were utilized for sensing of RDX and TNT. In addition, cilia from pig olfactory epithelia were also used for monitoring of cyclic adenosine monophosphate levels following exposure to odorants and explosives. This practical study implied that explosive substances as well as other odorants present in landmines interacted with olfactory receptors. Xavier *et al.* used a microfluidic device for OSN array [154]. By using calcium imaging, they could detect and analyze odorant responses of about 2900 OSNs for four different fruity/floral smells (vanilla, rose, berry, and banana) in microwells simultaneously. Their approach was based on a large-scale fluorescent investigation of many OSNs which were trapped in the micro chamber. For detection of cultured ORN responses *in vitro*, MEA equipped with gas intake system was also used by Ling *et al.* [80]. Limonene and isoamyl acetate odorants were tested in that system. They analyzed the firing spikes of ORNs and extracted and sorted the different spikes from multiple neuron recordings. Tanada *et al.* showed odorant sensor by means of expressing odorant receptors of insects into dissociated neural cultures of rats [135]. The hybrid system had advantages of easy functional expression of odorant receptors, prolonged lifetime, and amplification of weak ionic currents of odorant receptors.

As presented earlier, native ORN and OSN are directly usable as a biosensing device. Equally, host cells which could express different species' olfactory receptors have been employed for artificial odorant sensing. As a typical cell for such an expression system, a certain cell line of human embryonic kidney cells, namely, HEK293, is frequently used for the expression of several olfactory receptors. Using HEK293 cells, Ko and Park investigated the expression of rat olfactory receptor I7 (ORI7) [64] and showed that the HEK293 cells expressing ORI7 were usable for octanol detection through QCM [63]. Furthermore, Ko and Park reported that intracellular Ca^{2+} sensing molecule, yellow cameleon-2, could conjugate with olfactory receptors' response in HEK293 [65]. In 2009, Lee *et al.* presented that using planar microelectrodes, electrical signals could be obtained from HEK293 cells expressing ORI7 and the olfactory signals could be enhanced by electrical stimulation [74, 75]. In addition, they demonstrated real-time monitoring of cells' responses to odorant (heptanal, octanal, nonanal, decanal, and helional) stimuli using surface plasmon resonance (SPR) as shown in Figure 2.17 [76]. Oh *et al.* also used HEK293 cells expressing four different kinds of human olfactory receptors (hORs)—hOR3A1, hOR1A1, hOR1D2, and hOR1G1—for odorant screening [104]. They cultured the cells in polyethylene glycol diacrylate microwells and visualized the cells' response to odorants through fluorescent observation. Recently, Sato and Takeuchi demonstrated direct chemical vapor detection using HEK293 spheroids in hydrogel micro chambers [120]. As the transduction of cells' response to odorants, they used electrophysiological measurement. It is particularly worth noting that gas-phase odorants could be detected directly by HEK293 spheroids.

Some researchers focused on using host cells such as yeast, *Xenopus laevis* oocyte, and Sf21 cell derived from noctuid moth (*Spodoptera frugiperda*) for odorant sensing. Minic *et al.* successfully expressed ORI7 in budding yeast (*Saccharomyces cerevisiae*) and applied it to odorant screening [94]. They used luciferase reporter for detection of odorant binding events

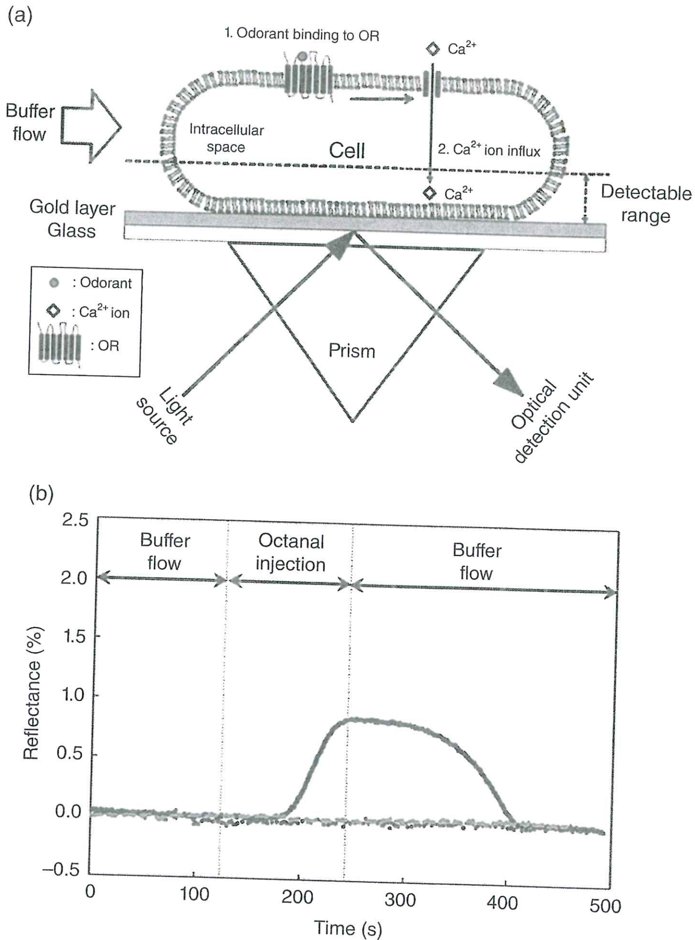


Figure 2.17 (a) Principle of SPR-based odorant detection using cells expressing odorant receptors. (b) SPR response to the odorant (octanol) stimulus. From Ref. [76]. Reproduced with permission from Elsevier

(Figure 2.18). Marrakchi *et al.* also used same species yeast expressing human olfactory receptor OR17-40 [90]. The yeasts were immobilized on integrated planar microelectrodes (Figure 2.19a) and the system measured the yeast conductance which reflected olfactory receptor activation by helional (Figure 2.19b). For explosive detection, genetically modified yeast was applied by Radhika *et al.* [109]. Coexpressed green fluorescent protein with rat olfactory receptor Olfr226 they newly identified was used as the probe for 2,4-dinitrotoluene detection. Misawa *et al.* showed that *Xenopus* oocytes expressing insect olfactory receptors could be sensor elements for odorant sensing [95]. They used small fluidic device integrated with electrodes for two-electrode voltage clamping. In that study, they demonstrated that the

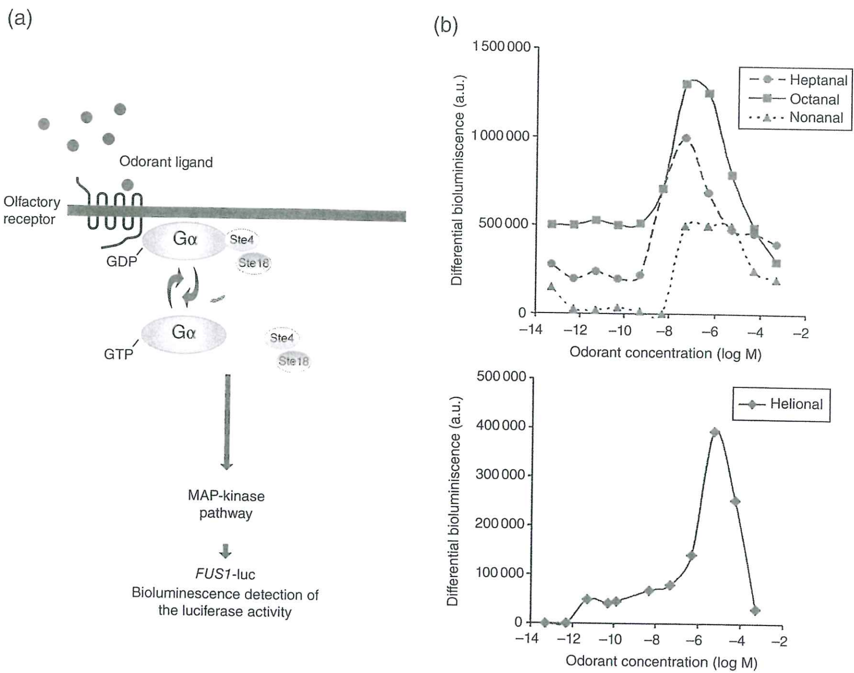


Figure 2.18 Odorant detection through a bioluminescence in genetically modified yeast. (a) Illustration of signal transduction pathway to yield odorant-induced luciferase activity. (b) Differential bioluminescence dose–responses on odorant stimulation. From Ref. [94]. Reproduced with permission from John Wiley & Sons, Ltd

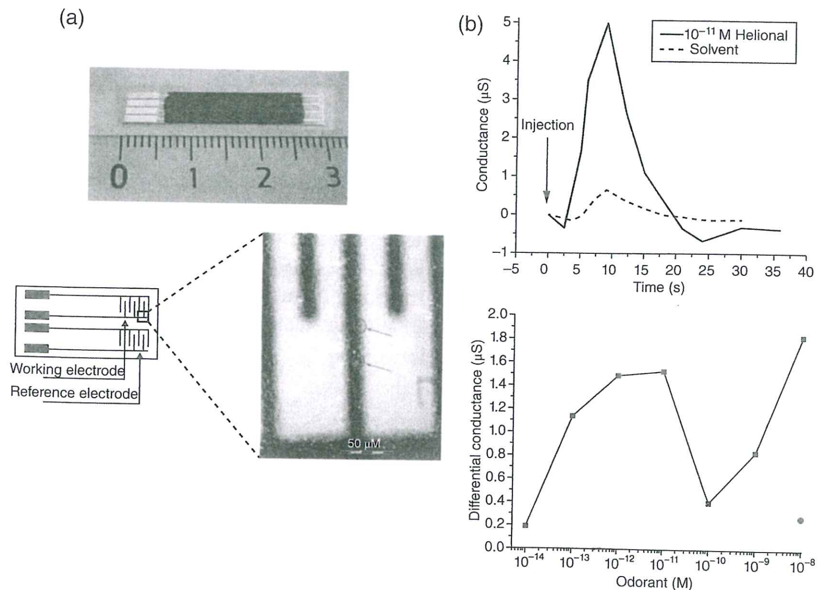


Figure 2.19 (a) Pictures and schematic presentation of interdigitated microelectrodes with deposited yeast cells. (b) The sensor signals and dose–response to helional. From Ref. [90]. Reproduced with permission from Springer Science +Business Media

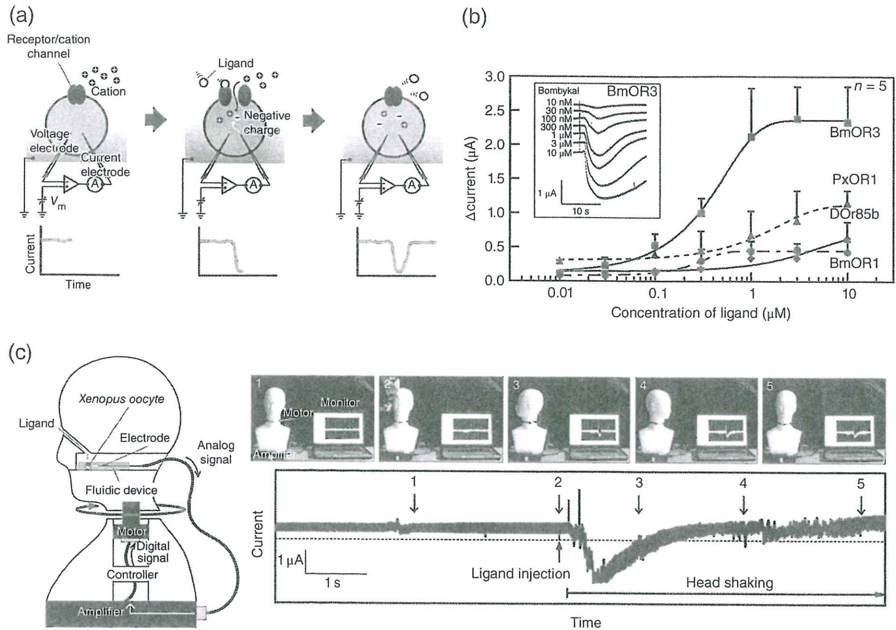


Figure 2.20 (a) Mechanism of TEVC-based odorant sensor using *Xenopus* oocytes expressing odorant receptors. (b) Dose-dependent increases in amplitude of each ligand-induced current. (c) Head-shaking robotic system with the cell-based odorant sensor device. From Ref. [95]. Reproduced with permission from National Academy of Sciences, USA

sensor device could be integrated with simple robotic system (Figure 2.20). Mitsuno *et al.* verified odorant detection using Sf21 cell lines coexpressing insect odorant receptors and Ca^{2+}-sensitive fluorescent protein named GCaMP3 (Figure 2.21) [96]. The Sf21 cell lines could express odorant receptors stably and detect odorants with consistent responsiveness for at least 2 months.

Concerning the variety of tested (or targeted) odorant compounds, it would appear that cell-based sensors are presently superior to tissue-based sensors as you can see in Table 2.2 As mentioned in the opening sentence, cell-based odorant sensors owe the wide range of object substance to accumulation of knowledge about olfactory mechanisms with advancing recent genetic technologies of expression systems.

2.4.3 *Receptor-Based Sensors*

As one type of olfactory biosensors, odorant receptor proteins themselves have been utilized as sensing elements for detecting target odorants. In general, this type of biosensors consists of functional odorant receptor proteins and transducers, which enable us to acquire

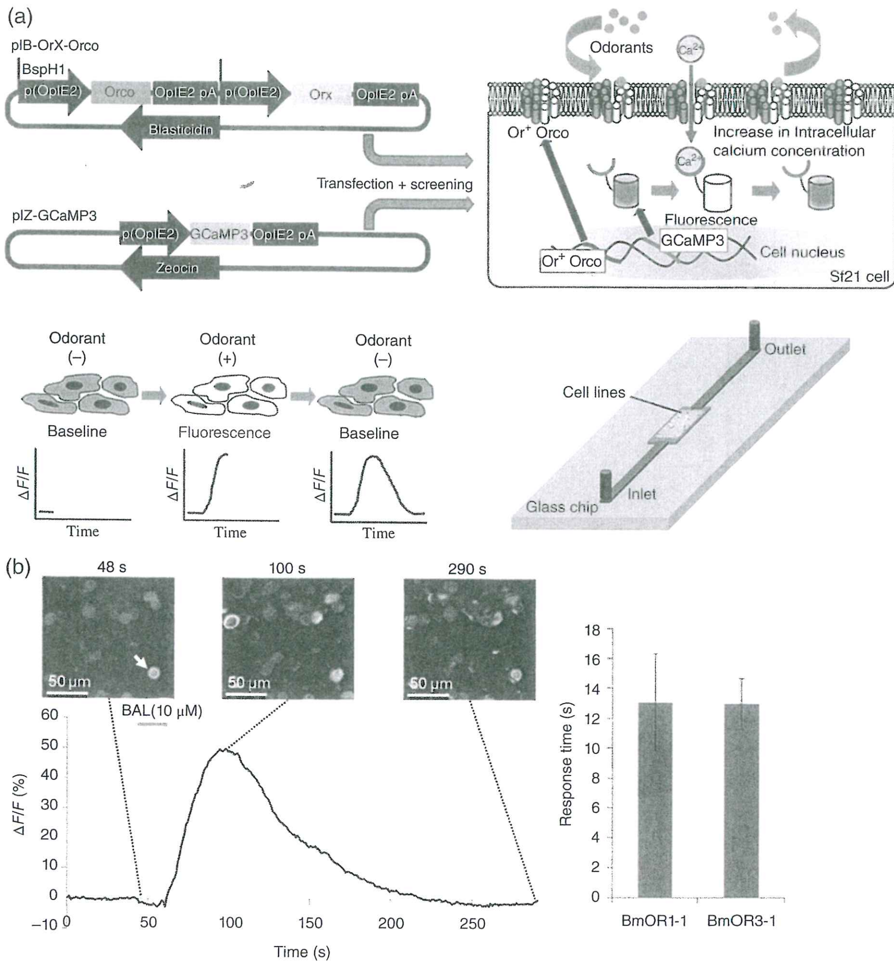


Figure 2.21 (a) Outline of odorant sensor using Sf21 cells expressing odorant receptors and Ca^{2+} sensitive fluorescent protein GCaMP3. (b) The fluorescent response and response time of cell lines. From Ref. [96]. Reproduced with permission from Elsevier

signals associated with interactions between odorant receptor proteins and odorants. Production of functional odorant receptor proteins (Figure 2.12) and their immobilization on the surface of transducers (Figure 2.13) are crucial for the development of receptor-based sensors. In this part, production and immobilization methods of various odorant receptors are summarized, and their application examples for biosensors are introduced.

Table 2.2 Summaries of cell-based sensors

Cell	Odorant receptor	Tested compound or ligand	Transducer	Tested concentration/ detection range	Literature
ORN (*Calliphora vicina*)	—	1,4-Diaminobutane, hexanol-1, butanoic acid	EAG	A few ppb to 500 ppm	[45]
ORN (*C. vicina*, *Aedes communis*, *H. abietis*, *Trogossita japonica*)	—	Hexanol, butyric acid, diaminobutane	EAG	0.1–100 μg	[46]
ORN	—	Acetic acid	LAPS	1–50 μM	[82]
OSN (Sprague-Dawley rat)	—	Mixture of acetic acid, octanol, cineole, hexanol, and 2-heptanone	LAPS	0.1 mM for each	[151]
OSN (pig and Sprague-Dawley rat)	—	TNT, RDX, amyl acetate, benzene, naphthalene, hexachloroethane, styrene, diphenylamine, benzothiazole, toluene, chlorobenzene	EOG and Ca^{2+}-related fluorescent detection (Fura-2/AM)	1 mM or 30 and 300 μM	[27]
OSN (Swiss-Webster mouse)	—	Vanillin, geraniol, benzyl acetate, ethyl butyrate	Ca^{2+}-related fluorescent detection (Fluo4-AM)	200 μM	[154]
ORN (Sprague-Dawley rat)	—	Limonene, isoamyl acetate	MEA	0.4–19 μM	[80]
Neuronal cell (Wistar rat)	BmOR1	Bombykol	Ca^{2+}-related fluorescent detection (EGFP)	1, 10, and 100 μM	[135]
HEK293	I7	Octanal	Ca^{2+}-related fluorescent detection (Fura PE3-AM)	0.1 μM to 10 mM	[64]
	I7	Hexanal, heptanal, octanal, nonanal, decanal	QCM	10^{-8} to 10 mM	[63]
	ODR-10, I7	Diacetyl, octanal	Ca^{2+}-related fluorescent detection (yellow cameleon-2)	0.1 and 1 mM	[65]
	I7	Octanal, helional	MEA	10 mM	[74]

	I7	Octanal	Planar electrode and Ca^{2+}-related fluorescent detection (Fura PE3-AM)	1, 5, and 10 mM	[75]
	I7	Heptanal, octanal, nonanal, decanal, helional	SPR	0.1, 1, 10, and 100 mM	[76]
	hOR3A1, hOR1A1, hOR1D2, hOR1G1	Helional, β-citronellol, bourgeonal, geraniol	cAMP-related fluorescent detection (ZsGreen)	50 nM to 500 μM	[104]
	GPROR2, Or47a	2-Methylphenol, benzaldehyde	Extracellular field potential recording	0.1 μM to 100 mM	[120]
Saccharomyces cerevisiae	I7, OR17-40	Heptanal, octanal, nonanal, helional	G_α subunit-related bioluminescence detection (FUS1-luc)	5×10^{-14} to 5×10^{-4} M	[94]
	OR17-40	Helional	Interdigitated microelectrodes	10^{-14} to 10^{-8} M	[90]
	I7, β_2AR, modified I7 for vanillin and citronellal (named VanR and CiteR), Olfr226	Octanol, octanal, heptanal, hexanal, isoproterenol, vanillin, citronellal, DNT	Ca^{2+}-related fluorescent detection (GFP)	1–50 μM	[109]
Xenopus oocyte	BmOR1, BmOR3, PxOR1, DOr85b	Bombykol, bombykal, A11-16:Ald, 2-heptanone	TEVC	0.01–10 μM	[95]
Sf21	BmOR1, BmOR3	Bombykol, bombykal	Ca^{2+}-related fluorescent detection (GCaMP3)	100–10 000 nM	[96]

2.4.3.1 Production of Odorant Receptors

For application of receptor-based biosensors, appropriate and functional odorant receptors need to be produced to be used as sensing elements for the detection of target odorants. Since the activity of odorant receptors affects various performances of biosensors, such as sensitivity, selectivity, and stability, the production of odorant receptors is an important step in the development of receptor-based biosensors. Several methods for production of odorant receptors have been reported: the extraction from olfactory organs (i.e., olfactory epithelium), overexpression in heterologous cell lines (i.e., bacteria, yeast, and mammalian cell line), cell-free production, and chemical synthesis (Figure 2.12).

Isolation of odorant receptors from the natural olfactory tissues of target living organisms is an effective method for the natural state of odorant receptors (Figure 2.12a). Since target odorant receptors are expressed in the ORNs, the odorant receptors are able to be isolated from olfactory tissues including ORNs. Wu showed that odorant receptors were able to isolate from the dissected olfactory epithelium of bullfrogs to coat onto a sensor array [150]. Since the advantage in this method is to maintain the natural structure of odorant receptors from native ORNs, isolated odorant receptors would exhibit molecular recognition to natural ligands. However, this method is inefficient for collection of large amount of odorant receptors, and it is difficult to isolate desired odorant receptors.

Heterologous expression systems have been commonly utilized for the production of large amount of desired odorant receptor (Figure 2.12b). In general, target odorant receptors genes are genetically subcloned into specific plasmid vectors to construct expression vectors for overexpression in heterologous cells. The expression vectors containing target odorant receptor genes are introduced into the heterologous cell by using transfection methods, such as lipofection, electroporation, microinjection, viral infection, and so on. In cells odorant receptors are yielded from the expression vectors, resulting in the collection of large amount of odorant receptors from the cells. So far, bacteria (*Escherichia coli*) [59, 131, 161], mammalian cell lines (human embryo kidney cell; HEK293 cell) [152, 153], and yeast (*S. cerevisiae*) [5, 6, 43, 124, 138, 139] have been often utilized as heterologous cells. This method has the following advantages: collection of large amount of desired odorant receptors, production of odorant receptors with odorant response profiles similar to in *vivo* profiles and purification of target odorant receptors with affinity tags. Therefore, various kinds of odorant receptors derived from vertebrates and invertebrates have been produced for analyzing their function and utilizing them as sensing elements by using this heterologous expression method [37]. However as disadvantages of this method, the codon usage of target odorant receptor genes might be considered for efficient expression, and proper heterologous cells might be required for getting active odorant receptors.

As an alternative method, cell-free protein production system is recently utilized for the production of membrane proteins containing odorant receptors (Figure 2.12c). Extracts from various living organisms such as wheat germ, *E. coli*, rabbit, and insect are commercially available. Since the extracts contain all cellular components for transcription and translation (RNA polymerase, ribosomes, tRNA, amino acids, energy source, and so on), target proteins are synthesized by mixing the extracts and expression vector containing a target gene *in vitro*. Kaiser *et al.* reported that the human odorant receptors were able to be produced by using cell-free production employing extract from wheat germ, demonstrating that the odorant receptors were available as sensing elements [51]. Similarly, Hamada *et al.* reported that silkmoth's pheromone receptor was synthesized in liposomes including the extract from

E. coli and expression vectors to produce functional odorant receptors [39]. This method as well as heterologous expression system yields and obtains large amount of heterologous proteins. However, there are a few cases for applications to biosensors.

Peptide synthesis is likely to be available as a method, for producing odorant binding sites of odorant receptors as sensing elements (Figure 2.12d). In this method, the binding sites in target odorant receptors were predicted by computational simulation and synthesized by peptide synthesis based on chemical reaction to obtain the sensing element for biosensor. Sankaran *et al.* have computationally simulated secondary structure from amino acid sequence of two mouse odorant receptors to predict odorant binding sites based on comparison to other receptor proteins and docking simulation. Based on the predicted binding sites, the polypeptides were chemically synthesized for sensing elements [118]. However, odorant binding sites of odorant receptors from vertebrates and invertebrates remain unclear. In the future, further knowledge regarding odorant binding sites in odorant receptors from various organisms could facilitate development of sensing elements utilizing polypeptides of odorant binding site.

2.4.3.2 Immobilization of Odorant Receptors

In order to acquire signals associated with interactions between odorant receptors and odorants, odorant receptors and their peptides need to be immobilized onto the surface of transducers. At this time, the odorant receptors have to be maintained on the surface at the state of native structure and with their odorant response profiles. So far, three types of methods are mainly used for immobilization of odorant receptors or their peptides onto the surface of transducers: physical adsorption, self-assembled monolayer (SAM) with biotin/avidin interaction, and Au—S bonding (Figure 2.13).

Physical absorption method has been commonly used for immobilization of odorant receptors onto the surface of transducers (Figure 2.13a). In this method, odorant receptor solution that is produced or isolated by the above methods (see Section 2.4.3.1) is generally spread on the electrode of the transducer (i.e., crystal electrode) without special coating materials, and the solution is completely dried in a desiccator. Due to simplicity of procedure, many researchers have utilized this method [59, 131, 150]. However, since unnecessary membrane proteins and other proteins were also absorbed, the biosensors' selectivity and specificity would be affected.

As a typical linker between solid surface and biomolecule, SAM of alkanethiol molecules has been widely used in the development of several biosensors. Since thiol group (—SH) strongly interacts with gold (Au), biomolecules can be easily immobilized on Au surface through the linker like "Au—S—$(CH_2)_n$—protein" using linear carbon chain whose one end is —SH and opposite end is carboxyl group or amino group, for instance. The distance between Au surface and the immobilized biomolecule is controllable due to SAM property of homogeneous thickness arising from hydrophobic interaction among the linear molecules that form the monolayer. Nowadays, there are many commercially available SAM reagents whose functional groups are preliminarily activated for tethering of biomolecules including some odorant receptors and the partial peptide chains such as odorant binding site. In addition, SAM that is combined with specific antibodies is able specifically and stably to immobilize desired odorant receptors. Therefore, SAM with specific antibodies is currently one of the methods that are often utilized for immobilizing odorant receptors onto the surface of transducers (Figure 2.13b). Odorant receptors with affinity tags are produced by using the heterologous

expression or cell-free production (see Section 2.4.3.1). The odorant receptors with tags are immobilized upon the surface of transducers that is coated by SAM with the antibodies for recognizing the tags. Vidic *et al.* reported that "nanosome" isolated from yeast expressing odorant receptors was immobilized on the gold surface of SPR device according to this method [138]. Hou *et al.* also successfully immobilized membrane fraction from yeast expressing an odorant receptor onto the electrochemical impedance spectroscopy (EIS) to measure the interaction between the receptor and odorants [43].

The methods utilizing Au—S bond were also used for immobilization of odorant receptors and polypeptides (Figure 2.13c). Amino acid sequences in proteins or odorant receptors include one type of amino acids, cysteine, whose chemical structure has a thiol group. A thiol group couples to Au to form strong bonds as described earlier. Utilizing this principle, odorant receptors and peptides are able to be fixed onto the surface of transducers. Sankaran *et al.* reported that the chemically synthesized polypeptide was immobilized by using this method [118].

2.4.3.3 Measurement from Odorant Receptors

Signals associated with interactions between odorant receptors and odorants have been measured by transducers, such as field-effect transistors (FET), EIS, QCM, SPR, and SAW. Receptor-based biosensors have been fabricated by various combinations of these transducers with various types of odorant receptors that were produced with the above methods. These examples are summarized in Table 2.3.

FET has been commonly used for acquirement of signals from purified odorant receptors. One of the important merits for using FET is to acquire weak signal of interaction between odorant receptors and odorants due to its innate signal amplification. In human odorant receptor-based bioelectronic nose, Kim *et al.* reported a single wall carbon nanotube (swCNT)-FET that was coated with human odorant receptors, hOR2AG1, which is selectively activated by amyl butyrate (Figure 2.22) [59]. Membrane fraction including the OR was collected from *E. coli* and immobilized onto swCNT-FET. The swCNT-FET sensor exhibited ultrahigh sensitivity at the scale of 100 fM and selectively detected amyl butyrate without detection of other similar chemicals. Similarly, Yoon *et al.* reported biosensors using carboxylated polypyrrole nanotubes (CPNT)-FET and hOR2AG1 [161]. They demonstrated that the chemical immobilization strategy with amino silane (3-aminopropyltrimethoxysilane) enabled them to maintain stable electrical contact in CPNT-FET and quantitatively control immobilization of hOR. The hOR-conjugated CPNT-FET sensor achieved high sensitivity to amyl butyrate at 40 fM and selective detection among similar chemicals. Not only odorant receptors but also peptides as sensing molecules were able to immobilize on the surfaces of swCNT-FET. Lim *et al.* successfully developed odorant receptor-derived peptide (ORP)-conjugated swCNT-FET, which sensitively and selectively detected trimethylamine in real time at concentration as low as 10 fM [77]. The ORP-conjugated swCNT-FET was also demonstrated to be able to determine the quality of three types of seafood and distinguish spoiled seafood.

EIS has been recently reported to be able to be used for odorant detection by immobilizing odorant receptors [2, 3, 43]. EIS consists of three electrodes: working electrodes (odorant receptors), reference electrodes, and counter electrodes. Hou *et al.* immobilized yeast-expressed ORI7 on a gold electrode (working electrode) by SAM and biotin/avidin system and detected odorant-dependent signal change by EIS [43]. They showed that the heptanal and

Table 2.3 Summaries of receptor-based sensors

Odorant receptors	Cell/production methods	Ligands	Transducer	Sensitivity/detection range/ detection limit	Literature
Isolated OR proteins (bullfrog)	Bullfrog (*Rana* spp.)/isolation	*n*-Caproic acid, isoamyl acetate, *n*-decyl alcohol, β-ionone, linalool, ethyl caproate	Piezoelectric crystal electrode	10^{-6} to 10^{-7} g (sensitivity)	[150]
hOR2AG1 (human)	Bacteria (*Escherichia coli*)/cell based	Amyl butyrate	swCNT-FET	100 fM (detection limit)	[59]
	Bacteria (*E. coli*)/cell based	Amyl butyrate	CPNT-FET	40 fM (detection limit)	[161]
OR17-40 (human), ORI7 (rat)	Yeast (*Saccharomyces cerevisiae*)/cell based	Helional, cassione, piperonyl acetate, 3,4-methylenedioxyphenylacetone, 3,4-methylenedioxypropiophenone (OR17-40), octanal (ORI7)	SPR	Approx. 5×10^{-10} to 5×10^{-6} M (detection range (figure))	[138]
OR17-40 (human)	Yeast (*S. cerevisiae*)/cell based	Helional	SPR	—	[139]
	Yeast (*S. cerevisiae*)/cell based	Helional	SPR	10^{-11} to 10^{-5} M (tested range)	[5, 6]
OR17-4 (human)	Extract from wheat germ/ cell-free	Undecanal	SPR	1.2–100 μM	[51]
ORI7 (rat)	Yeast (*S. cerevisiae*)/cell based	Octanal, heptanal, helional	EIS	10^{-13} to 10^{-4} M (detection range)	[43]
	Yeast (*S. cerevisiae*)/cell based	Octanal, helional	EIS	—	[2–4]
ODR-10 (*C. elegans*)	Bacteria (*E. coli*)/cell based	Diacetyl (2,3-butanedione)	QCM	10^{-12} to 10^{-5} M (detection range)	[131]
	Human breast cancer cell (MCF-7)/cell based	Diacetyl (2,3-butanedione)	SAW	1.2×10^{-11} mM (detection limit), 10^{-10} to 10^{-4} mM (detection range)	[152, 153]
Polypeptides (OR binding sites)	Chemical synthesis	Acetic acid, butyric acid, ammonia, dimethylamine, chlorobenzene, benzene	QCM	0.9 mg/ml to 108 mg/ml (acetic acid)	[87]
	Chemical synthesis	1-hexanol, 1-pentanol	QCM	2–3ppm (1-hexanol), 3–5ppm (1-pentanol) (detection limit)	[119]
	Chemical synthesis	Trimethylamine	swCNT-FET	10 fM to 1 μM (100 μM)	[77]

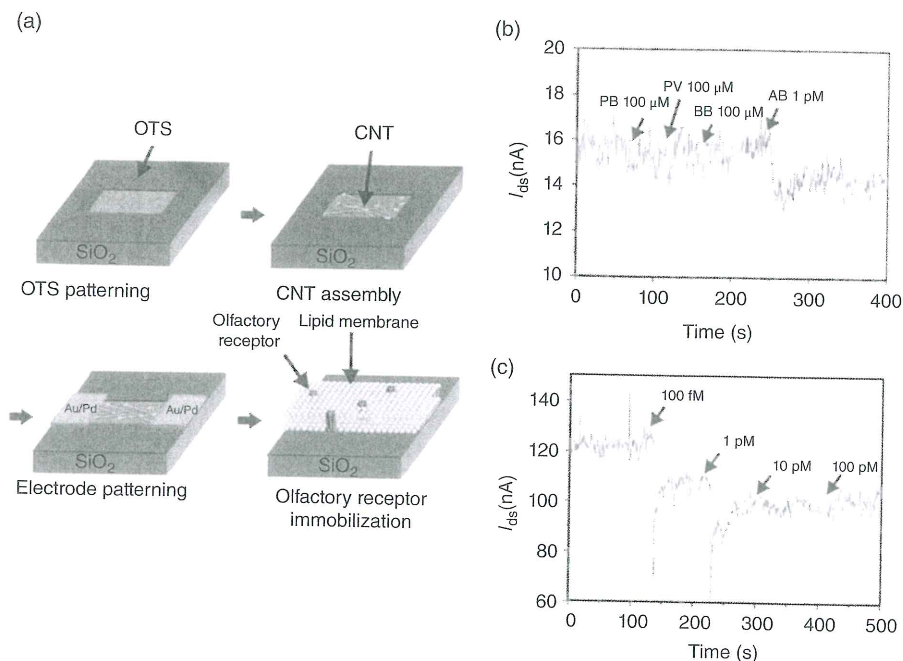
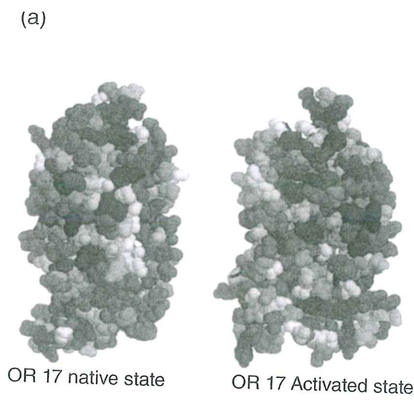
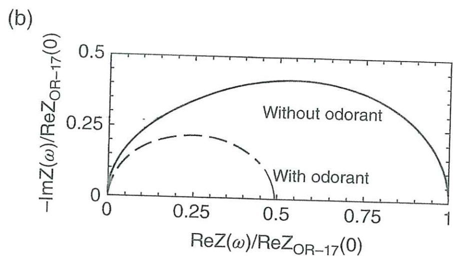


Figure 2.22 Human odorant receptors (hOR2AG1)-functionalized swCNT-FET sensor. (a) Fabrication of hOR2AG1-functionalized swCNT-FET sensor. OCT, octadecyltrichlorosilane. (b) Odorant selectivity of the swCNT-FET sensor. AB, amyl butyrate; BB, butyl butyrate; PB, propyl butyrate; PV, pentyl valerate. (c) Responses of the swCNT-FET sensor to indicate concentrations of AB. From Ref. [59]. Reproduced with permission from John Wiley & Sons, Ltd

octanal were successfully detected using functionalized electrodes. More recently, Alfinito *et al.* showed that the differences in polarization resistance associated with inactive and ligand-bound states of rat receptor ORI7 were measured using EIS and the presence or absence of odorants was analyzed by Nyquist plot (Figure 2.23) [3]. From this theory, they described that electrical properties of a single sensing protein are one possibility to be available as sensing elements.

QCM has been commonly used for odor sensors. Signals of odorant receptors can be obtained from QCM-coated odorant receptors. Sung *et al.* reported that crude membrane extracts of *E. coli* expressing *Caenorhabditis elegans* odorant receptor, ODR-10, were coated onto the surface of QCM by physical absorption (Figure 2.24). Odorant receptors (ODRs) in *C. elegans* belong to GPCR family as same as mammalian odorant receptors. The sensors were demonstrated to selectively detect diacetyl (2,3-butanedione), which is a ligand of ODR-10, with a dynamic range of 10^{-12} to 10^{-5} M [131]. Sankaran *et al.* combined QCM and polypeptides that were chemically synthesized based on computational simulation for prediction of odorant binding sites [118]. This biosensor was developed to detect 1-hexanol and 1-pentanol with detection limits of 2–3 and 3–5 ppm, respectively, which could be applied into the detection of bacterial pathogens in packaged beef. Similarly, a QCM sensor





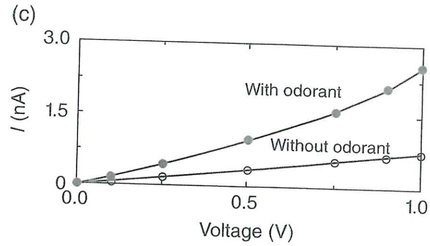


Figure 2.23 (a) Conformational changes of rat ORI7 in the native and activated state. (b) Nyquist plot of the ORI7 with or without the odorant. (c) *I–V* characteristics of the ORI7 with or without the odorant. From Ref. [3]. Reproduced with permission from Elsevier

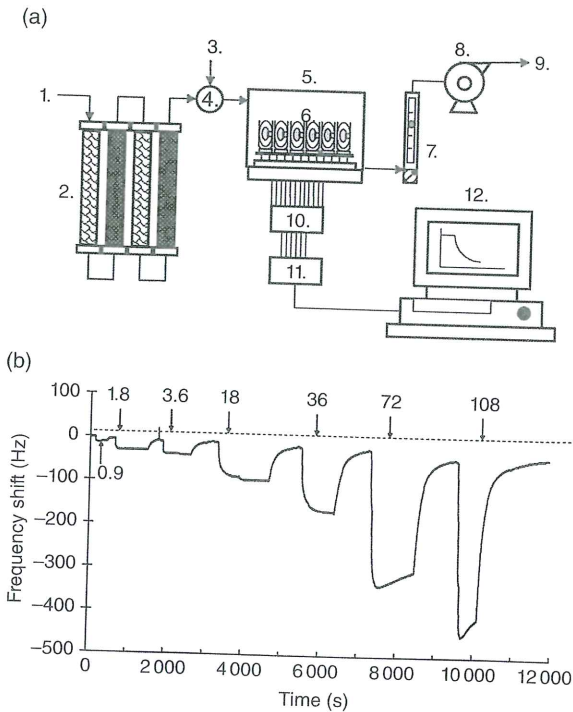


Figure 2.24 (a) Schematic drawing of QCM biosensor. (1) Carrier gas inlet, (2) silica gel and activated carbon, (3) sample inlet, (4) three-way valve, (5) detection chamber, (6) multiarray sensors, (7) flow meter, (8) vacuum pump, (9) waste, (10) oscillator circuit, (11) frequency counter, and (12) computer. (b) Frequency shifts of the sensing system to various concentrations of acetic acid. From Ref. [87]. Reproduced with permission from Elsevier

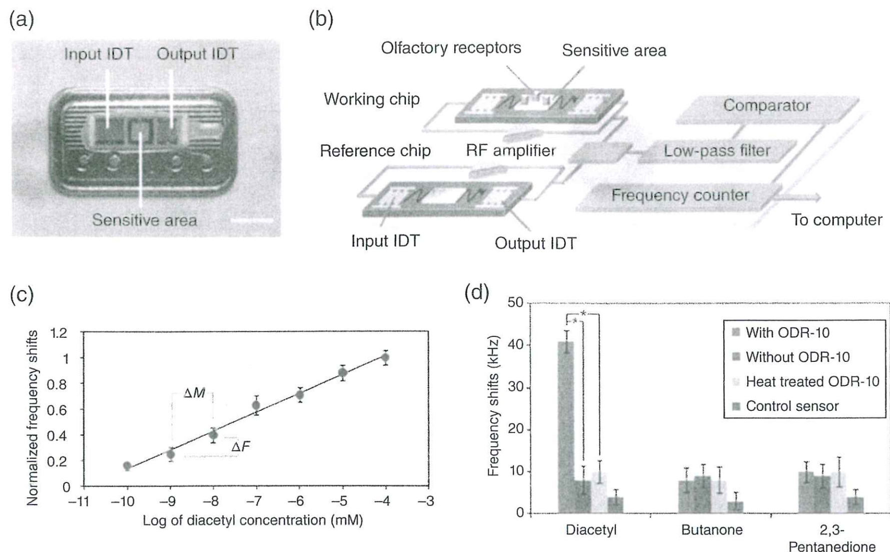


Figure 2.25 SAW device. (a) A photograph of a SAW chip. IDTs, interdigital transducers. Scale bar: 5 mm. (b) Schematic diagram of measurement system of odorant receptor (ODR10a)-functionalized SAW chip. (c) Dose–responses of the SAW chip to various concentrations of diacetyl. (d) Responses of SAW chip immobilized with various membranes to different odorants. From Ref. [153]. Reproduced with permission from Elsevier

combined with chemically synthesized OBP was developed to detect alcohols at room temperature, and the estimated detection limit was about 1–3 ppm [119]. In addition, Lu *et al.* developed a six-chip sensor module with QCMs containing synthetic polypeptides together with conducting polymers to achieve simultaneous detection and identification of various classes of VOCs [87].

SAW sensors were also demonstrated to be possible to be utilized as transducers for measuring signals from odorant receptors (Figure 2.25). Wu *et al.* reported that the SAW sensor-coated membrane fraction of human breast cancer cells, MCF-7 cells, expressing ODR-10 exhibited 10-fold more sensitive (10^{-13} M) to natural ligand, diacetyl, than those of QCM sensors [152]. Furthermore, they demonstrated the SAW sensors with better performances by improving the immobilization efficiency of the odorant receptors onto the surface of SAW chip with SAM absorption to achieve higher sensitivity (10^{-15} M) and longer stability (within 7 days) than their previous work (Figure 2.25) [153].

SPR-based sensing was one of the most popular methods to detect several biomolecules' interactions. Vidic *et al.* showed that nanosomes prepared from OR-expressed yeast were used for detecting odorants through a SPR [138]. In this work, rat ORI7 or human OR1740 with Gα protein was coexpressed in yeast, *S. cerevisiae*, and the yeast-derived nanosomes were immobilized with carboxymethyl-modified dextran polymer hydrogel on the surface of SPR sensor chip. Under the condition of GTPγS existence, which is nonhydrolyzable analogue of GTP, the detection of odorants was achieved by measuring the amount of mobilization of

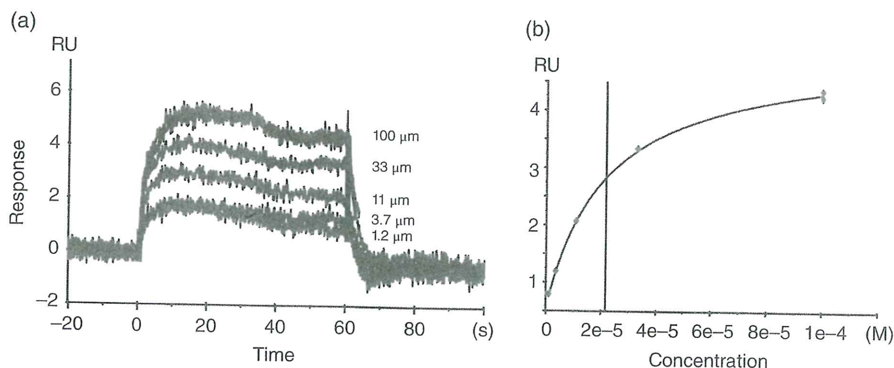


Figure 2.26 Detection of odorant interaction with hOR17-4 on surface plasmon resonance. (a) Responses to undecanal at indicated concentrations. (b) Dose-dependent curve. From Ref. [51]. Reproduced with permission from National Academy of Sciences, USA

Gα protein along with GTPγS. According to this method, helional and cassione were selectively detected in a dose-dependent manner. Vidic *et al.* also demonstrated the immobilization method of nanosome on the gold surface of SPR device by SAMs and biotin/neutravidin to construct nanosome patterning using microcontact printing [139]. In reports of SPR for OR-based biosensors, it was also reported that cell-free produced odorant receptors were applicable (Figure 2.26) [51]. Human odorant receptors, hOR17-4, were synthesized using wheat germ cell-free protein production system and immobilized on a sensor chip of SPR apparatus, Biacore T100 (GE Healthcare). The OR-conjugated SPR sensor dose-dependently detected undecanal, which is known as a ligand of hOR17-4.

2.4.4 Summary of the Biosensors

As mentioned in these earlier sections, several odorant sensors based on living systems (bion, organ, tissue, cell, and receptor) have been developed in recent years. There are currently many application studies of human odorant receptors for such a biosensing. On the one hand, some insect odorant receptors are being used as the odorant sensor elements these days. Behind the trend is the fact that insect olfactory systems can be partly reconstructed at cellular level with the recent progress in revealing of the olfactory mechanism. In addition, peptides whose structural motifs have been predicted as odorant binding site are also applied to odorant sensors recently.

Optical-based, resonance-based, and electrical device-based detection systems are mainly used as transducers between the biomaterials and the artificial output interfaces. The combination of these transducers and biomaterials provides many variations of odorant biosensors. Each odorant biosensor has advantages and disadvantages as summarized in Table 2.4. Although each approach has actually some difficulties such as lifetime and laborious handling process of biomaterials, they possess their own advantages in points of the sensitivity and the target odorants as shown in each table of the respective sections. In other words, any odorant biosensors have not yet led to realize the versatile feature of sensitivity,

Table 2.4 Advantages and disadvantages of odorant sensors

Types of biosensor	Advantages	Disadvantages
Tissue-based sensor	Ease to fabricate and immobilize Natural odorant profiles Low cost	Lack of specificity Individual differences Olfactory fatigue Difficulty of natural state and storage Need to kill animals
Cell-based sensor	High sensitivity and selectivity Single type of ORs Nature of membrane for ORs	Difficulty of handling Necessity of culturing Low stability Difficulty of long-term sensing
Receptor-based sensor	High sensitivity and selectivity Longer-term stability Acquirement of receptor activity	Difficulty of purification and isolation

selectivity, portability, robustness, cost-effectiveness, and so on. However, it is expected that further understanding of creature's olfactory system will facilitate a development of odorant biosensors with better performance.

2.5 Future Prospects

According to the elucidation of molecular mechanisms in olfaction of natural living organisms, the mechanism can be used for the olfactory biosensors based on biological molecules. In this chapter, three different types of olfactory biosensors have been introduced: tissue-based biosensor, cell-based biosensors, and receptor-based biosensors. Some researches demonstrated that the performances of these biosensors were superior to those of existing odor sensors in terms of sensitivity and selectivity. However, the olfactory mechanisms of living organisms have been not completely elucidated. Living organisms detect and discriminate various types of environmental odorants with higher performances than expected. For example, recent research demonstrates that biological molecules, such as OBPs, coreceptor, membrane proteins, and membrane transport proteins, function in their olfactory organs and ORNs. In the future, along with the advances in studies on olfaction and taste, the performances of artificial odorant biosensors reach to those of living organisms.

Response characteristics in olfactory biosensors are in accordance with those of the expressed odorant receptors. Therefore, in order to develop odorant biosensors for detecting target odorants, we need to characterize the odorant selectivities of various types of odorant receptors from various living organisms to select the odorant receptors that enable us to detect a target odorant. The database of odorant selectivity in odorant receptors has been constructed regarding to *D. melanogaster* odorant receptors and mammalian odorant receptors. We can use them to select the odorant receptors for the target odorants. The methodology we have described in this chapter, in conjunction with the large repertoire of odorant receptors, enable to develop practical odorant biosensors for various applications, such as food administration, environmental monitoring, and health management.

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