Chapter 18

Bioelectronic Nose Using Olfactory Receptor-Embedded Nanodiscs

Heehong Yang, Minju Lee, Daesan Kim, Seunghun Hong, and Tai Hyun Park

Abstract

Olfactory receptors (ORs) are the largest family of the G protein-coupled receptors (GPCRs), which are significantly involved in many human diseases and 40% of all drug targets. A platform containing stable and high-quality OR would be a powerful tool for the development of a practical biosensor that can be applied to various applications, such as the early diagnosis of diseases, assessment of food quality, and drug and fragrance development. Significant efforts have been made to develop the biosensor using GPCRs; nevertheless, they remain a challenge. This chapter describes an attractive methodology for the development of a stable bioelectronic nose using OR-embedded nanodiscs. The ORs were produced in *Escherichia coli* (*E. coli*), purified with column chromatography, reconstituted into nanodiscs and applied to a carbon nanotube-field effect transistor (CNT-FET) with floating electrodes. The nanodisc-based bioelectronic nose exhibits high-performance in terms of sensitivity, selectivity and stability. This strategy can be used as a practical method for the receptor-based sensing approach, which represents significant progress in nano-bio technology toward a practical biosensor.

Key words Olfactory receptor, Bioelectronic nose, Nanodisc, Carbon nanotube, Field-effect transistor

1 Introduction

Olfactory receptors (ORs), which belong to the G protein-coupled receptors (GPCRs), are transmembrane proteins that are comprised of seven α-helical domains [1]. In humans and mammals, ORs play an important role in distinguishing and recognizing thousands of odors [2, 3]. Some ORs are very specific to certain odorants, while others have broad specificity [3, 4]. These characteristics are very useful to develop bioelectronic sensors for various applications, such as fragrance development, diagnosis of disease, food safety, and disaster response [5, 6]. Purified and well-reconstituted ORs, especially, have the great advantage of mimicking the olfactory sensory response [7, 8]. For this reason, many

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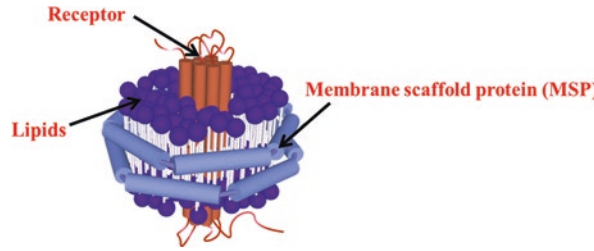

Fig. 1 Schematic diagram of olfactory receptor-embedded nanodiscs. The nanodiscs consist of a receptor, lipids, and membrane scaffold protein (MSP). The MSP tightly wraps the edge of the lipid bilayer; thus, the receptor-embedded nanodiscs can be stable in an aqueous environment and mimic the native structure of receptors in the intact cells

researchers have studied the purification and functional reconstitution of OR; however, they remain a challenge due to their complicated structure and strong hydrophobicity [9–11]. Among the many reconstitution techniques, such as detergent micelles, liposomes, nanodiscs and lipid cubic phases, nanodiscs are considered the most appropriate tool for GPCR reconstitution for various applications [9, 12, 13]. Nanodiscs consist of a receptor, lipid bilayer, and membrane scaffold protein (MSP) (Fig. 1) [14]. The lipid bilayer provides the receptor with a well-organized biomimetic environment and the MSP wraps around the hydrophobic edge of the lipid, which can stabilize the receptor in an aqueous environment as well as a dried condition [15].

Recently, the bioelectronic sensors based on carbon nanotubes (CNTs) have been developed to monitor the responses of ORs to their odorants. Previously, it has been reported that the introduction of floating electrode structures in carbon nanotube field effect transistors (CNT-FETs) enhanced the sensor performance [16, 17]. Also, due to the gold floating electrodes, nanodiscs can be conveniently immobilized on the gold with an appropriate orientation by using simple linkers [18].

In this chapter, we present the production and fabrication protocols for bioelectronic nose using OR-embedded nanodiscs. For the production of OR and MSP, *Escherichia coli* (*E. coli*) was used due to its productivity and convenience. The OR-embedded nanodiscs have a native-like binding pocket, which leads it to mimic the response of a natural olfactory receptor. The CNT-FET with floating electrodes converts the olfactory responses of the nanodiscs to electrical signals with high sensitivity. The nanodisc-based bioelectronic nose has advantages in terms of reliability and reproducibility as well as sensitivity and selectivity. Moreover, this methodology facilitates the development of highly stable biosensors, which can be utilized for practical applications.

2 Materials

2.1 Production of Olfactory Receptor-Embedded Nanodiscs

1. Host cell: BL21 (DE3) competent *E. coli* cells at 37 °C in a shaking incubator.
2. Cell culture medium: Luria–Bertani (LB) medium supplemented with 50 μg/mL ampicillin.
3. Cell culture flask: Erlenmeyer flask (2 L).
4. OR-cloned expression vector: Fuse a ribosome binding site (RBS) sequence (*see* **Note 1**) at the N-terminus of OR gene (*see* **Note 2**). Insert the RBS-OR gene into pET-DEST42 vector (*see* **Note 3**). Prepare 200 ng/μL of pET-DEST42/RBS-OR plasmid.
5. MSP-cloned expression vector: Fuse a ribosome binding site (RBS) sequence (*see* **Note 1**) at the N-terminus of the MSP gene, and a 6 × His-tag sequence (*see* **Note 4**) at the C-terminus of the MSP gene (*see* **Note 5**). Insert the RBS-MSP-6xHis gene into the pET-DEST42 plasmid. Prepare 200 ng/μL of pET-DEST42/RBS-MSP-6xHis plasmid.
6. Isopropyl thiogalactoside stock solution (IPTG) (*see* **Note 6**): 1 mM of IPTG in distilled water.
7. 10 K MWCO dialysis cassette.
8. 0.2 μm bottle top filter.
9. Columns: HisTrap HP column, HiTrap HP desalting column and size exclusion chromatography (SEC) (Superdex 200 Increase 10/300 GL).
10. Solubilization buffer: 0.1 M Tris–HCl, 20 mM sodium dodecyl sulfate (SDS), 100 mM dithiothreitol (DTT), 1 mM EDTA, pH 8.0.
11. Washing buffer 1: 0.1 M sodium phosphate, 10 mM SDS, pH 8.0.
12. Lysis buffer: 20 mM Tris–HCl, 0.5 M NaCl, 20 mM imidazole, pH 8.0.
13. Washing buffer 2: 20 mM Tris–HCl, 50 mM imidazole, 0.5 M NaCl, pH 8.0.
14. Elution buffer: 20 mM Tris–HCl, 450 mM imidazole, 0.5 M NaCl, pH 8.0.
15. BCA assay kit (Pierce, USA).
16. Lipids: palmitoyloleoylphosphatidylcholine (POPC) and palmitoyloleoylphosphatidylglycerol (POPG).
17. Bio-Bead.

2.2 Fabrication of a Nanodisc-Functionalized Bioelectronic Nose

1. Silicon wafer with 3000 Å SiO_2 layer.
2. Semiconducting single walled carbon nanotube (ssCNT) dispersion: Prepare ssCNT dispersion to the final concentration of 0.05 mg/mL in 1,2-dichlorobenzene. Use an ultrasonic cleaner for 5 h.
3. Octadecytrichlorosilane (OTS) solution: Disperse 1:500 v/v OTS in anhydrous hexane.
4. Photoresist AZ5214, DNR.
5. N-acetyl-L-cysteine (NAC) solution: 0.5 M of NAC in distilled water.
6. V5 tag monoclonal antibody.
7. 2-mercaptoethylamine hydrochloride (2-MEA) solution: Prepare 12 mg/mL 2-MEA in a PBS buffer with 2 mM EDTA solution. Store at 4 °C.

2.3 Measurement of Olfactory Responses

1. HEPES buffer 1: 20 mM HEPES–NaOH, 100 mM NaCl, pH 8.0.
2. HEPES buffer 2: 20 mM HEPES–NaOH, 100 mM NaCl, 25 mM cholate, 1 mM EDTA, pH 8.0.
3. HEPES buffer 3: 20 mM HEPES–NaOH, 100 mM NaCl, 1 mM EDTA, pH 8.0.
4. Odorants: Prepare 1 M odorant stock solutions with DMSO. Serially dilute the odorant stock solutions with HEPES buffer 1 and store them at 4 °C.
5. Measuring equipment: 4200 semiconductor analyzer and Probe station.

3 Methods

3.1 Expression and Purification of Olfactory Receptor

1. Transform the BL21(DE3) competent cells with the pET-DEST42/RBS-OR vector.
2. Culture the transformed cells in the cell culture medium at 37 °C until the OD_{600} value reaches 0.6.
3. Induce the production of the OR by adding IPTG.
4. Incubate the cells for 4 h.
5. Centrifuge the cultured cells (7000 × *g*, 30 min, 4 °C).
6. Resuspend the pellets of the sample in PBS with 2 mM EDTA.
7. Disrupt the cells by sonication (5 s on/off, 5 min) and centrifuge the cell lysate (12,000 × *g*, 4 °C, 30 min).
8. Repeat the sonication and centrifugation, and solubilize the pellet with solubilization buffer at 25 °C overnight.

9. Dialyze the solubilized proteins against 0.1 M sodium phosphate (pH 8.0) containing 10 mM SDS using a 10 K MWCO dialysis cassette.
10. Filtrate the sample with a 0.2 μm bottle top filter.
11. Apply the protein sample to the HisTrap HP column equilibrated in 0.1 M sodium phosphate (pH 8.0) containing 10 mM SDS using fast protein liquid chromatography (FPLC).
12. Wash successively with a gradient (pH 8.0–7.0) using the washing buffer 1 and elute the OR with the same buffer at pH 6.0.
13. Dialyze the purified OR against the HEPES buffer 2 and store it at 4 °C.

3.2 Expression and Purification of MSP

1. Perform the same procedures in Subheading 3.1, **steps 1–4** using the pET-DEST42/RBS-MSP-6 × His expression vector.
2. Resuspend the pellets with a lysis buffer and disrupt the cells by sonication (5 s on/off, 5 min).
3. Centrifuge the cell lysate (12,000 × *g* for 30 min at 4 °C).
4. Filtrate the MSP in the supernatant with a 0.2 μm bottle top filter and apply to HisTrap HP column using FPLC.
5. Wash the column with the washing buffer 2.
6. Elute the MSP with the elution buffer.
7. Dialyze the purified MSP against the HEPES buffer 2 using the HiTrap HP desalting column and store at 4 °C until used.
8. Analyze the purified OR and MSP by SDS-PAGE and western blot analysis.
9. Measure the concentrations of purified proteins using a BCA assay kit.

3.3 Formation of Olfactory Receptor-Embedded Nanodiscs

1. Mix the POPC and POPG at a 1:1 molar ratio and dry them using nitrogen gas from a chloroform solution.
2. Vacuum the lipids for 1 h to remove the residual chloroform.
3. Solubilize the dried lipids with HEPES buffer 2 (18 mg/mL) and store at −80 °C until used.
4. Sonicate the lipids with bath sonication for 15 min and successively freeze and thaw it 3 times. Then, repeat the procedures.
5. Add the prepared lipids to a purified OR, and then incubate them for 10 min in ice.
6. Add the MSP to the mixture, and incubate the mixtures containing OR, lipids and MSP for 2 h at 4 °C by stirring (The final concentrations: 1 μM OR, 100 μM MSP, 8 mM lipids and 25 mM cholate).

7. Add the Bio-Bead to the mixtures to remove the detergents with overnight agitation.
8. Load the samples to a size exclusion chromatography (SEC) (Superdex 200 Increase 10/300 GLequilibrated with HEPES buffer 3 using FPLC to remove unbound proteins.
9. Collect the peak fractions and store the purified OR-embedded nanodiscs at 4 °C.

3.4 Fabrication of a CNT-FET with a Floating Electrode

1. Coat a silicon wafer with AZ5214 using a spin coater. Operate the spin coater with 900 × *g* for 55 s. Prebake the wafer at 95 °C for 7 min.
2. Place the wafer and an align key mask on a photolithography aligner. Expose the wafer to UV light. Dip the wafer in a developer solution. Rinse the wafer with distilled water and dry it by nitrogen gas (Fig. 2).
3. Deposit 10 nm titanium (Ti) and 30 nm gold (Au) layers on the wafer using thermal evaporation.
4. Lift off the Ti/Au layer on the photoresist layer by immersion in acetone for more than 1 h. Rinse the wafer with acetone and ethanol. Dry the wafer by nitrogen gas.
5. Repeat **step 1**, and place the wafer and a CNT pattern mask on the aligner. Expose the wafer to UV light. Dip the wafer in the developer solution. Rinse the wafer with distilled water and dry it by nitrogen gas.
6. Immerse the wafer in OTS solution at 80% humidity for 6 min (*see* **Note 7**). Rinse the wafer with anhydrous hexane. Remove the photoresist layer of the wafer with acetone. Rinse the wafer with acetone and ethanol. Dry the wafer using nitrogen gas.
7. Immerse the wafer ssCNT dispersion for 20 s (*see* **Note 8**). Rinse the wafer with 1,2-dichlorobenzene and dry the wafer using nitrogen gas.

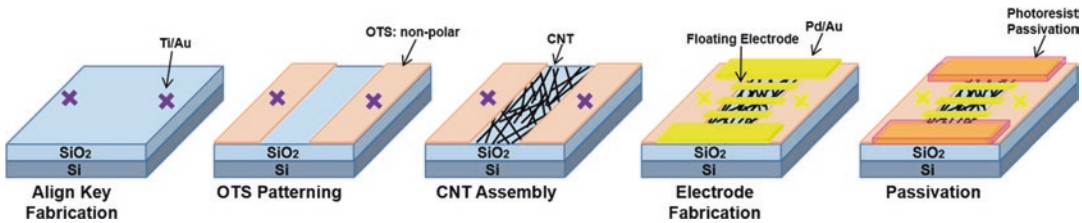

Fig. 2 Schematic diagram representing the fabrication processes of a CNT-FET with floating electrodes. First, an align key pattern (Ti/Au) was fabricated on a bare SiO_2 wafer. An OTS self-assembled monolayer with non-polar terminal groups was patterned on the SiO_2 wafer, followed by the selective assembly of CNTs. Then, source, drain, and floating electrodes were fabricated by photolithography and thermal deposition. Lastly, the source and drain electrodes were covered with a photoresist passivation layer

8. Repeat **step 1**, and place the wafer and an electrode pattern mask on the aligner. Expose the wafer to UV light. Dip the wafer in the developer solution. Rinse the wafer with distilled water and dry it by nitrogen gas.
9. Deposit 10 nm palladium (Pd) and 15 nm Au layers on the wafer using thermal evaporation.
10. Lift off the Pd/Au layer on the photoresist layer by immersion in acetone for more than 1 h. Rinse the wafer with acetone and ethanol. Dry the wafer by nitrogen gas.
11. Coat the silicon wafer with DNR using the spin coater. Operate the spin coater with 4000 rpm for 55 s. Prebake the wafer at 95 °C for 100 s.
12. Place the wafer and a passivation pattern mask on the aligner (*see* **Note 9**). Expose the wafer to UV light.
13. Bake the wafer at 115 °C for 100 s.
14. Dip the wafer in the developer solution. Rinse the wafer with distilled water and dry it by nitrogen gas.
15. Bake the wafer at 90 °C by using a hot plate. Raise the temperature of the hot plate by 10 °C per 10 min. At 200 °C, sustain the temperature for about 8 h.

3.5 Immobilization of Nanodiscs on a CNT-FET with Floating Electrode

1. Mix 1 μL of V5 Ab solution with 49 μL of 2-MEA solution for 1.5 h at 37 °C (*see* **Note 10**).
2. Incubate the prepared CNT-FET in NAC solution for 10 min at 37 °C for the channel functionalization with the thiol groups (*see* **Note 11**).
3. Slightly rinse the channel with distilled water, and then gently blow the CNT-FET with nitrogen gas.
4. Treat the half-fragments of V5 Ab on the NAC-functionalized CNT-FET channel for 1 h at room temperature.
5. Slightly rinse the channel with PBS, and then gently blow the CNT-FET with nitrogen gas.
6. Place 1 μL of nanodisc solution on the channel. Incubate the CNT-FET with the nanodisc solution for 30 min at room temperature (Fig. 3).
7. Rinse with HEPES buffer 1 to remove unbound nanodiscs.

3.6 Measurement of Olfactory Responses Using Bioelectronic Nose

1. Develop the buffer zone of 9 μL onto the channel of the bioelectronic nose to monitor the responses of the olfactory receptors using the bioelectronic nose.
2. Fix the bioelectronic nose on the vacuum chamber of the probe station.
3. Maintain the drain–source bias voltage at 0.1 V on the bioelectronic nose.

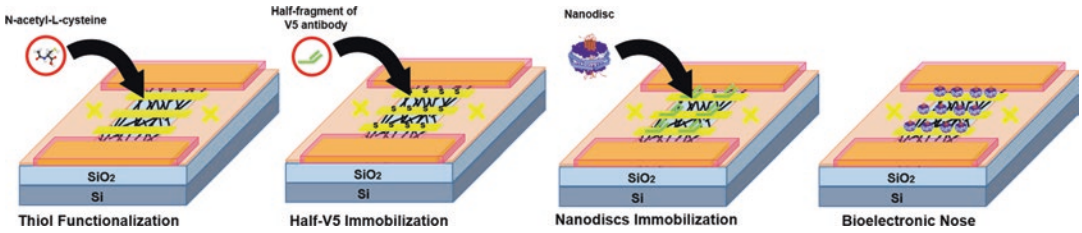

Fig. 3 Schematic diagram of the immobilization of nanodiscs on the floating electrodes of the CNT-FET. The Au surfaces of the floating electrodes were functionalized with thiol groups. Then, half-fragments of the V5 antibody were immobilized. Lastly, olfactory receptor-embedded nanodiscs were immobilized for the fabrication of a bioelectronic nose

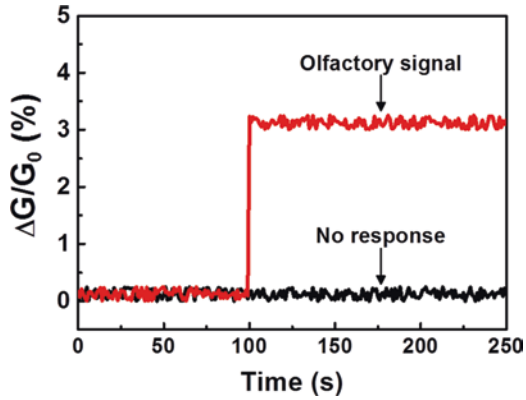

Fig. 4 Schematic diagram representing olfactory signal responses of the bioelectronic nose. When the odorant–receptor interaction occurred, the olfactory signal responses were visible by monitoring the current change of the bioelectronic nose

4. Add the 1 μL of odorant solution onto the buffer zone from low concentrations to high concentrations by the factor of 10 each time.
5. Monitor the change of drain–source current of the bioelectronic nose during the addition of the target solutions (Fig. 4) (*see* **Note 12**).

4 Notes

1. The pET-DEST42 bacterial expression vector does not contain the RBS site, thus the researcher should design and insert the RBS site at the N-terminus of the OR gene for protein expression. In here, the RBS sequence (5' AGGAGATATACAT 3′) was designed and used.
2. The vertebrate OR genes do not contain an intron site; thus, they can be simply amplified using a genomic DNA mixture by polymerase chain reaction (PCR) [19].

3. The pET-DEST42 vector contains V5 epitope and 6xHis genes at the C-terminus of the cloning site. It also contains LacO and LacI genes, which are used to operate the protein expression.
4. The MSP needs a 6xHis-tag for purification, but should not contain a V5 epitope-tag for oriented immobilization of the nanodiscs on a floating electrode of CNT-FET. Thus, we inserted the 6xHis sequence at the C-terminus of the MSP gene.
5. Nanodiscs consist of an OR, lipid bilayer, and membrane scaffold protein (MSP). The MSP wraps around the hydrophobic edge of the lipid, which can stabilize nanodiscs in an aqueous environment and also in dried condition [15]. The representative MSP is apolipoprotein A-I (ApoA-I), which is the major protein component of high density lipoprotein particles in plasma [20]. In this method, the ApoA-I gene was amplified by the PCR using human genomic DNA.
6. The production of protein using the pET-DEST42 expression vector was operated with LacO and LacI genes. Normally, the LacI protein would block the expression of protein in *E. coli* cells. The expression of protein was induced by the addition of 1 mM IPTG.
7. An OTS self-assembled monolayer with nonpolar terminal groups was patterned on the wafer for selective ssCNT assembly. Because of the hydrophobic property of OTS, ssCNTs cannot be attached to the OTS pattern [21].
8. The ssCNTs are selectively absorbed on the bare SiO_2 layer. The ssCNT device exhibits p-type semiconducting characteristics [22].
9. Source and drain electrodes are covered with the passivation layer for the elimination of leakage currents during measurement in liquid environments.
10. A V5 antibody splits to a couple of half-fragments of the V5 antibody which are linker proteins for the immobilization of the nanodiscs. The one end of the half-fragment of the V5 antibody is bound to the thiol-functionalized surface, and the other is combined with the V5 epitope of the nanodiscs [18].
11. Thiol groups are functionalized on an Au surface via the incubation process in the NAC solution.
12. When an odorant binds to OR-embedded nanodiscs, the structure of the OR changes, which affects the conductance of the FET [23, 24]. Thus, the interaction of the OR with a specific odorant can be measured by monitoring the conductance change in real time.

Acknowledgments

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