

PORTABLE MEMBRANE PROTEIN CHIP:

DEVELOPMENT OF MEMBRANE PROTEIN SENSORS FOR ENVIRONMENT ANALYSIS

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ABSTRACT

This paper describes a portable measurement system using membrane proteins (receptors) for environment analysis (Figure 1). Creatures sense ambient stimulations as eyesight, smell, taste, and so on, using recipient cells with the receptors in the cell membrane. If those congenital sensing mechanism is exploitable in an artificial system, the bio-inspired system will be applied for ultrahigh sensitive and selective sensors. For developing this sensor, we have addressed the stable and the reliable bilayer lipid membrane (BLM) as a platform for the membrane proteins. Double-well chamber with the parylene micropore that has the Ag/AgCl electrodes on the bottom of the chamber allows us to make the portable BLM device. As the results, we demonstrate the channel current recordings of the membrane protein using our portable BLM system at an extreme environment (at the summit of Mt. Fuji, Figure 2a). This proves that our system can be brought the higher mountain and measured the signals in wild environments.

KEYWORDS

Membrane proteins, Lipid bilayer, Biosensors, Portable system, MEMS

INTRODUCTION

Ion channels and biological nanopores have been studied toward the practical application in such as drug discovery, next generation of DNA sequencing, and biological sensors.^[1] Especially, the bio-inspired sensing based on the receptors which receipts environment substances is recently studying as an ultrahigh sensitive sensor. Bilayer lipid membranes (BLMs) are usually formed across small apertures opened in a hydrophobic support.^[2] BLMs are often formed with either the painting method or the Montal-Muller (MM) method. In the painting method, a lipid solution (an organic solvent containing phospholipid) is applied across a tiny aperture that separates two aqueous compartments. In the MM method, lipid monolayers at the water–air interface are brought together when they are

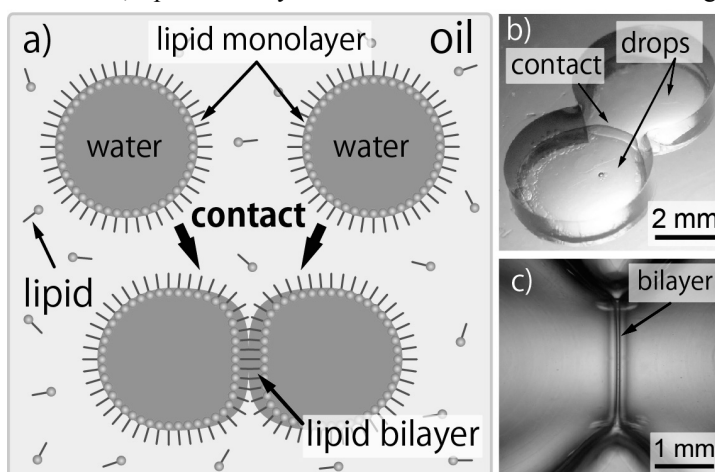


Figure 1 a) Illustration of the droplet-contacting method. The monolayer assembles spontaneously at the interface between water and the organic solvent containing amphiphilic molecules. Once the two interfaces come into contact with each other, they form a lipid bilayer. b) A photograph of the double-well chip (DWC). Two aqueous drops are contacted each other. c) Enlargement photograph of the interface of DWC. Lipid monolayer on the droplet surfaces will form the bilayer at the surface. This method should be one of the easiest ways for preparing the lipid bilayer.

raised above the aperture. Planar bilayers facilitate the study of membranes in precisely defined environments—for instance, investigations of buffer composition at both the *cis* and *trans* sides and membrane potentials. The electrical seal-resistance of the BLMs system is superior to that of the cell patch-clamping system, allowing highly sensitive detection, ideally down to single molecular level. However, bilayers produced using the conventional methods are often fragile, unsteady, and difficult to reproduce; this reduces their usefulness in high-throughput systems for pharmaceutical screenings.

Our group has recently developed a reproducible method, the “droplet-contacting method (DCM)” for forming planar bilayers, using a simple fluidic control.^[3] The principle of this approach is shown in Fig. 1a. The monolayer assembles spontaneously at the interface between water and the organic solvent containing amphiphilic molecules (phospholipids; Fig. 1a). Once the two interfaces come into contact with each other, they form a lipid bilayer. This method can easily be performed by injecting two water droplets into a well that is already filled with lipid solution. The success of the technique can be verified by capacitance measurements made between the membrane and ion channel signals, through peptide channels reconstituted into the membrane. Fig. 1c shows the top view of the contacted membranes. The interface does not rupture when an organic solution containing lipid molecules is used; the two droplets stay unmixed for over an hour. Thus, this system is both straightforward and stable.

In addition, we also have previously reported rapid cocaine sensing by using DNA aptamer and the protein channel.^[4] However, there are several issues in the membrane protein sensing for the environment analysis. The issues to be solved are: 1) the current noise; the particular equipment are required because 10^{-12} A current are measured in the channel recordings. 2) the stability of BLM; BLM usually are broken with artifacts caused by vibrations. 3) the preparation of BLM with membrane proteins; the BLM formation requires proficient skills. To solve these issues, we developed a low noise portable patch-clamp amplifier (PICO, Tecella) with BLM chip which makes stable BLM by DCM. This system will lay the groundwork for the real-time, point-of-care sensing of a wide variety of environmental targets.

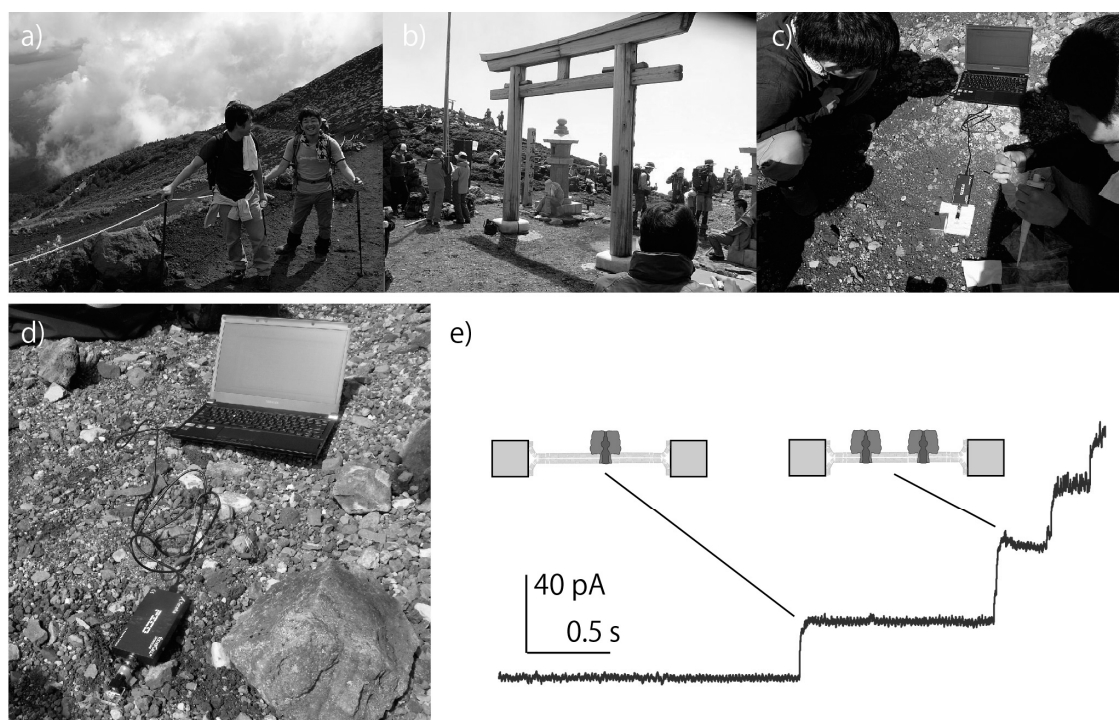


Figure 2 a) A photograph of the climbing Mt. Fuji for measuring the channel current using the portable BLM system. b) At the summit of Mt. Fuji (3776 m). c,d) The BLM chip was connected to the handheld patch-clamp amplifier (PICO, Tecella), and the single channel current was able to be measured with low noise at the extreme environment. e) The current-time trace of α HL channel recordings at the summit using the portable system. The recording was conducted using DPhPC/n-decane, 1 M KCl, 10 mM PBS, 1 mM EDTA buffer at 30 mV bias voltage. The single channel conductance of α HL was around 1 nS under this condition.

EXPERIMENT

We previously used a DWC for the droplets contacting method and prepared BLMs. However, we found the BLMs prepared by this method were mechanically unstable, which resulted in membrane rupture in relatively short time. In this study, we attempted to confine the BLMs forming areas with parylene micro-pores to enhance the mechanical stability (parylene double well chip, PDWC).^[5] A fabricated parylene film which has five pores (150 μm in diameter,) was sandwiched between PMMA films and separated the two round chambers.

The PDWC with electrode was fabricated for the handheld recordings of channel proteins. Ag/Cr was deposited and patterned on the PMMA plate as wired electrodes for electrical recording from the chambers to a portable patch-clamp amplifier. Then, the chambers with parylene films and the wired plate were connected by thermocompression bonding. Finally, the bottoms of the chamber which contacts droplets were coated with Ag/AgCl paste. BLMs formation process is as follows; each well was filled with *n*-decane containing a phospholipid and two droplets of buffer solution were dropped into lipid solution in each well. A few minutes later, BLMs were spontaneously formed at parylene micropores.

RESULTS AND DISCUSSIONS

To prepare BLM, two droplets in oil/lipid mixture first make lipid monolayers on the surfaces and then the monolayers are attached together to form a bilayer as shown in Fig. 1a-c. This method would be the easiest one for preparing BLM. The BLM chip was integrated with the parylene micropore and connected to the handheld patch-clamp amplifiers (Pico, Tecella) through the electrodes (Fig. 2a). The BLMs in the micropore was robust to the mechanical vibration, especially in low frequency. In addition, the current noise was reduced by changing the electrodes position from top to bottom of the chamber. Additionally, if BLM is ruptured, it can be reawaked by repainting at the interface between the wells. The success rate for the α -hemolysin (α HL) reconstitution by using the repainting methods was better than that by the first drop-off. Time required for the BLM formation by the repainting was more than 2 times faster than that by the drop-off method. Finally, we successfully measured the single channel currents of α HL using our handheld system at the summit of Mt. Fuji (Fig. 2b).

CONCLUSION

The portable BLM system for the recording of the protein channel currents can actually use in the extreme environment. This system will be applied to the ultrahigh sensitive bio-inspired sensors for environment analysis.

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