Faraday Discussions

Lateral voltage as a new input for artificial lipid bilayer systems

Journal:	*Faraday Discussions*
Manuscript ID	FD-ART-07-2021-000045.R1
Article Type:	Paper
Date Submitted by the Author:	25-Aug-2021

Complete List of Authors:	Ma, Teng; Tohoku University, Advanced Institute for Materials Research (WPI-AIMR) Sato, Madoka; Tohoku University, Research Institute of Electrical Communication Komiya, Maki; Tohoku University, Research Institute of Electrical Communication Kanomata, Kensaku; Yamagata University, Graduate School of Science and Engineering Watanabe, Takaya; Tohoku University, Research Institute of Electrical Communication Feng, Xingyao; Tohoku University, Research Institute of Electrical Communication Miyata, Ryusuke; Tohoku University, Research Institute of Electrical Communication Tadaki, Daisuke; Tohoku University, Research Institute of Electrical Communication Hirose, Fumihiko; Yamagata University, Graduate School of Science and Engineering Tozawa, Yuzuru; Saitama University, Graduate School of Science and Engineering Hirano-Iwata, Ayumi; Tohoku University, WPI-Advanced Institute for Materials Research; Tohoku University, Research Institute of Electrical Communication

Lateral voltage as a new input for artificial lipid bilayer systems

Teng Ma,[ab] Madoka Sato,[cd] Maki Komiya,[c] Kensaku Kanomata,[e] Takaya Watanabe,[cd] Xingyao Feng,[c] Ryusuke Miyata,[cd] Daisuke Tadaki,[c] Fumihiko Hirose,[e] Yuzuru Tozawa,[f] and Ayumi Hirano-Iwata*[abc]

[a] Advanced Institute for Materials Research (WPI-AIMR), Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577 Japan
[b] Core Research Cluster, Tohoku University, 2-1-1 Katahirano, Aoba-ku, Sendai 980-8577 Japan
[c] Research Institute of Electrical Communication, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577 Japan
[d] Graduate School of Biomedical Engineering, Tohoku University, 6-6 Aoba, Aramaki, Aoba-ku, Sendai 980-8579, Japan
[e] Graduate School of Science and Engineering, Yamagata University, 4-3-16 Jonan, Yonezawa 992-8510, Japan
[f] Graduate School of Science and Engineering, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama-shi, Saitama 338-8570, Japan
*ayumi.hirano.a5@tohoku.ac.jp

Abstract:

In this work, we propose lateral voltage as a new input for use in artificial lipid bilayer systems in addition to the commonly used transmembrane voltage. To apply a lateral voltage to bilayer lipid membranes, we fabricated electrode-equipped silicon and Teflon chips. The Si chips could be used for photodetector devices based on fullerene-doped lipid bilayers, and the Teflon chips were used in a study of the ion channel functions in the lipid bilayer. The findings indicate that the lateral voltage effectively regulates the transmembrane current, in both ion-channel-incorporated and fullerene-incorporated lipid bilayer systems, suggesting that the lateral voltage is a practicable and useful additional input for use in lipid bilayer systems.

Introduction

The bilayer lipid membrane (BLM) is an essential component of the cell membrane. It is formed via the self-assembly of amphiphilic lipid molecules at water/water interface. The first artificial free-standing BLM was reported by Mueller et al., who painted a solution containing lipids on a hydrophobic aperture.[1] Since then, artificial BLMs have been widely accepted as a feasible platform for studies of the biological functions of cell membranes.[2–5] After 60 years of intensive research on artificial BLM systems, techniques for preparing BLMs have greatly evolved. For example, the "folding" method was proposed to reduce the oil (non-volatile solvent) residue that is present in free-standing membranes;[6] and the droplet interface bilayer (DIB) method was developed to form a stable BLM in an oil bath.[7,8] In our group, we also adopted the well-established micro-fabrication process used in semiconductor technology to form silicon (Si) chips with a nano-tapered aperture, on which stable and strong oil-free BLMs could formed.[9,10] Based on the Si chip, we incorporated various ion channel proteins into BLMs and investigated drug-related side effects on cardiac ion channels.[11,12] Such BLM systems are now being applied to a wider range of fields. For instance, nanomaterial-doped BLMs have been demonstrated to have great potential in studies related to self-assembled devices for different applications, such as light sensing devices and water desalination.[13–16] In previous studies, we also doped BLMs with fullerene derivatives, and fabricated self-assembled devices based on hybrid membranes composed of lipids and fullerene derivatives.[17,18] The devices demonstrated reversible and repetitive responses to light illumination.

In contrast to versatile progresses in BLM platforms, the measurement approach of artificial BLM systems has not changed substantially in the past several decades. In a typical BLM system, both sides of a BLM are filled with buffer solutions; and a transmembrane voltage is applied to the BLM through two electrodes that are immersed in the buffer solution. The transmembrane voltage is usually used as the input, and the measured transmembrane current is used as the output of the system. This simple single-input, single-output system can provide valuable information in terms of

understanding the basic functions of membrane proteins, and for developing functional biosensors and nano-bio hybrid devices.[2,19,20]

Inspired by the evolution from two-terminal diodes to three-terminal transistors in semiconductor technology, we expected that introducing an additional input to a BLM system might allow better control over the system and additional information could be obtained from the system, which may help to create new types of lipid membrane platforms that do not exist in nature. In this work, we propose a method to improve the control of the BLM system by applying a voltage parallel to the BLM (lateral voltage) in addition to the traditional transmembrane voltage (Figure 1). To apply the lateral voltage to artificial BLM systems, we fabricated two different types of electrode-equipped membrane supports based on Teflon films and Si chips. The resulting BLMs were then functionalized with ion channel proteins and nanomaterials. The effects of the lateral voltage on both the ion-channel-incorporated and the nanomaterial-doped BLM systems were evaluated.

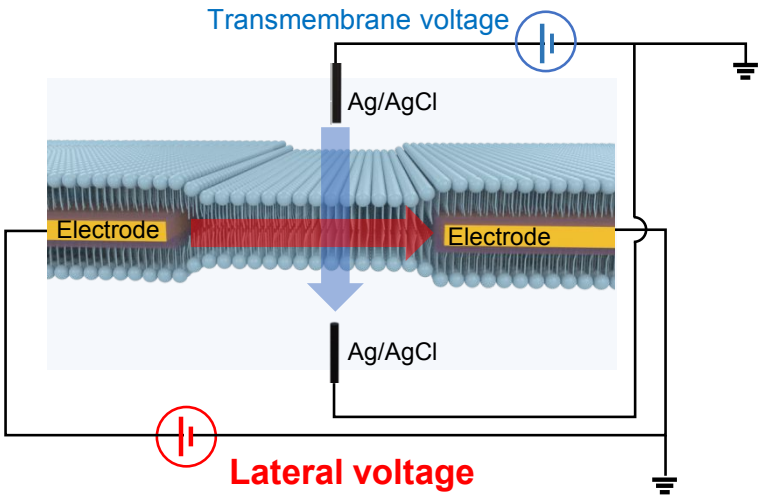

Fig. 1 Conceptual scheme for the two inputs (lateral and transmembrane voltages) BLM system.

Experimental

In order to apply a lateral voltage to the BLM system, we needed to fabricate an electrode-equipped membrane support for the free-standing BLM. In previous studies, we used micro-fabricated Si chips as membrane supports to form BLMs and applied

them to producing biosensors and light sensing devices.[10,17,21] On the other hand, Teflon films have traditionally been used as a support for BLMs in functional analyses of ion channel activities.[6,22,23] In this study, we developed suitable processes for fabricating electrodes on these Teflon films and Si chips.

For the Si chips, we previously reported on the use of mask deposition to form electrodes for the lateral voltage applications.[18] The simplified fabrication process is shown in Figure 2, a single crystal silicon substrate with a Si_3N_4 coating was first thermally oxidized to form a SiO_2 layer (Figure 2(a,b)); Si was then selectively etched by a photolithography process (Figure 2(c,d)); after which, the chip was thermally oxidized to form a protective SiO_2 coating (Figure 2(e)); photolithography was then used to selectively remove the SiO_2 to form a circular window at the center of the Si chip (Figure 2(f)); the underlying Si_3N_4 and SiO_2 were then sequentially etched to form a center aperture in the Si chip (Figure 2(g,h)); we then used thermal evaporation to form aluminum (Al) electrodes though a metal mask (Figure 2(i)); To prevent the buffer from causing the corrosion of the electrodes, we formed a SiO_2 protective layer on the electrodes (Figure 2(j)). Note that not all of the Al surfaces were covered by SiO_2, the uncovered parts of the Al electrodes were used to connect to external circuits for the application of a lateral voltage. Finally, the back side of the chip was coating with CYTOP® (AGC) to reduce the capacitance of the Si chip system and to reduce system noise (Figure 2(k)).[10] Before BLM formation, the surface of the electrode-equipped Si chip was subjected to treatment with a long-chain perfluorocarbon, (tridecafluoro-1,1,2,2-tetrahydrooctyl) dimethylchlorosilane (PFDS).[24]

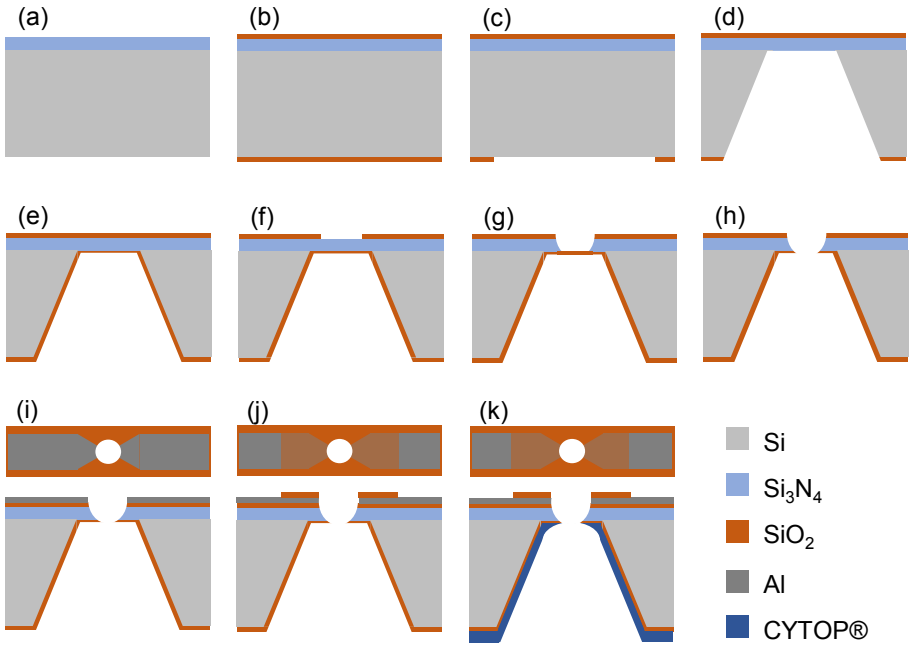

Fig. 2 Process used to fabricate the electrode-equipped Si chip for the application of lateral voltages.

To fabricate an electrode-equipped Teflon chip, we formed a circular aperture (~100 μm diameter) on a 12-μm-thick Teflon film by electrical discharge method (Figure 3(a,b)).[25] On the top of the film, an electron-beam (EB) evaporation technique was used to deposit titanium (Ti) electrodes (Figure 3(c)). To ensure that the lateral voltage is effectively applied to the BLM, the Ti electrodes extended to the very edge of the aperture. Similar to a Si chip, to protect the electrodes from buffer corrosion, we formed a protective SiO_2 layer on the surface of the electrodes via EB evaporation (Figure 3(d)). Finally, to prevent the oxidation of the Ti surfaces that were exposed to the atmosphere, we deposited a layer of Pt on the exposed Ti electrodes (Figure 3(e)). The resulting Teflon chip is shown in Figure 3(f). Before BLM formation, the SiO_2 surface was subjected to silanization with PFDS in the same manner as was applied to Si chips.

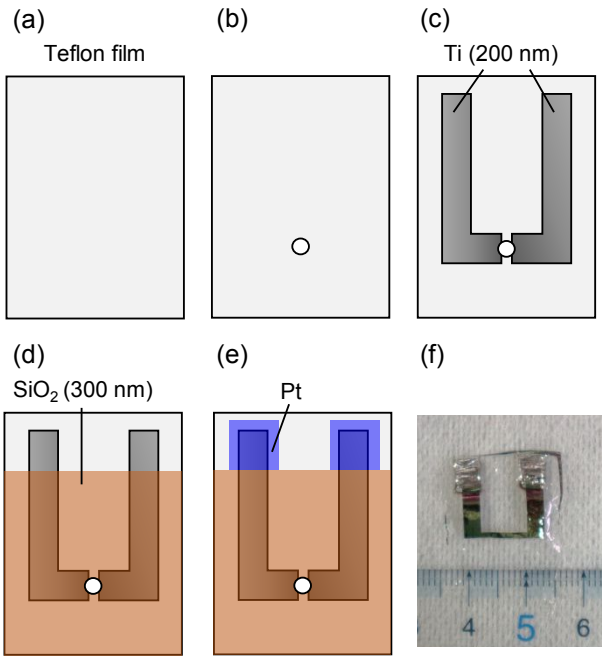

Fig. 3 Process used in the fabrication of the electrode-equipped Teflon chip for the application of lateral voltages.

Both the DC and AC voltage sources that were used for the application of lateral voltages were generated by homemade circuits with batteries as power sources to minimize hum noise. The AC voltage source has a sinusoidal waveform with a frequency of 11.6 kHz.[18]

The electrode-equipped Si chip was used for producing of BLMs doped with a fullerene derivative as a light-sensitive membrane. 1,2-Diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) and phenyl-C_{61}-butyric acid methyl ester (PCBM) were dissolved in chloroform/n-hexane to form a solution with a molar ratio of 5:1 (DPhPC:PCBM). The concentration of the DPhPC was 10 mg/mL. Solvent-free BLMs doped with PCBM were formed by the folding method.[17,18] Two electrodes on the Si chip, used as the lateral electrodes, were connected to the AC voltage source. Two Ag/AgCl electrodes were placed in buffer solution surrounding BLMs, on which transmembrane voltages were applied. Lights from a light emitting diode (525 nm, Solis High Power LED, Thorlabs) were used to illuminate the hybrid membrane.

The electrode-equipped Teflon chip was used for BLMs that were incorporated with

human voltage-gated sodium channel $Na_V1.5$ that was prepared from human embryonic kidney (HEK) 293T cells expressing the channel. HEK 293T cells (DS Pharma Biomedical Co., Ltd.) were cultured in Dulbecco's modified Eagle's medium/GlutaMAX medium supplemented with 10% fetal bovine serum (GIBCO). One to two days before the protein extraction, the cells were transfected with a plasmid, Nav1.5(SCN5A) (NM_198056) Human Untagged Clone (ORIGENE), using PEI MAX reagent (Polysciences, Inc.) and OPTI-MEM medium (GIBCO). Proteoliposomes containing human $Na_V1.5$ were isolated according to the procedures as previously described.[11] All the steps were performed at 4 °C. Briefly, cells obtained from a 100 mm plate were rinsed with HBSS (GIBCO) and scraped off into a 0.9 mL solution of 200 mM KCl, 33 mM KF, 10 mM EDTA, and 50 mM HEPES (pH 7.4 with KOH) plus a protease inhibitor cocktail (EDTA-free) (NACALAI TESQUE, INC.). The cells were homogenized and spun at 2000×*g* for 10 min. The supernatant was spun again at 4000×g for 20 min. The membrane fractions were pelleted from the supernatants by centrifugation at 157000×g for 1 h and resuspended in a 4:1 (v/v) mixture of a Na_V recording solution and glycerol. Na_V recording solution contained 149.2 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, and 5.0 mM HEPES-NaOH (pH 7.3). The obtained samples were stored at -30 °C until used. BLMs were formed across the apertures in the electrode-equipped Teflon chip in the same manner as previously described.[10], except that the edge of the aperture had been pre-coated with n-hexadecane before the buffer solutions were added to the two compartments of a Teflon recording chamber. The $Na_V1.5$ channels were then incorporated into the BLM by fusing proteoliposomes containing the channels with the BLMs.[12]

The transmembrane current was recorded using a Axopatch 200B patch-clamp amplifier (Molecular Devices). Signals were filtered with a low-pass Bessel filter, digitized, and stored online using a data acquisition system (Digidata 1440A and1550B, and pCLAMP, Molecular Devices). For the BLMs doped with PCBM, the cut-off and sampling frequency was 1 and 10 kHz, respectively. For the BLMs integrated with $Na_V1.5$ channels, the cut-off and sampling frequency was 2 and 25 kHz, respectively.

Results and discussion

Construction of lateral-voltage-equipped BLM system

To investigate the influence of additive layers (metal electrodes, SiO_2) of the BLM support on BLM quality, we formed free-standing BLMs on electrode-equipped Si chips and Teflon chips and evaluated their electrical characteristics. The resistance of the BLMs formed on the Si chip and the Teflon chip both exceeded the upper limit of the measuring instrument (200 GΩ), indicating that the additional electrodes and SiO_2 had no measurable effect on the BLM formation. We also confirmed that there was no leakage current observed between the two electrodes in buffer solutions and after BLM formation, indicating that the SiO_2 protective layer on the chip surface effectively protected the metal electrodes from the corrosion by the buffer solution.

The effect of lateral voltage, including DC and AC voltages, on BLM stability and transmembrane current noise were next investigated using membranes containing only lipids. Applying the lateral AC voltage to the Si chip, as previously reported, caused no noticeable effect on the stability and noise level of the system.[18] When a lateral voltage of 1 V (peak to peak) was applied, the resistance remained above 200 GΩ. Moreover, the current noise was as low (~3 pA) as that of BLMs in the absence of a lateral voltage (Figure 4(a)). However, when applying a DC voltage to the Si chip, we observed that the resistance of the BLM was reduced to about 100 GΩ, and the noise level increased significantly from 3 pA to over 80 pA, which is not suitable for current recordings at a pA level (Figure 4(a)). When a higher DC voltage (2 V) was applied to the lateral electrodes, electrochemical corrosion was observed at the edge of the lateral electrodes, indicating that the SiO_2 coating on the Si chip could not withstand such a high DC lateral voltage.

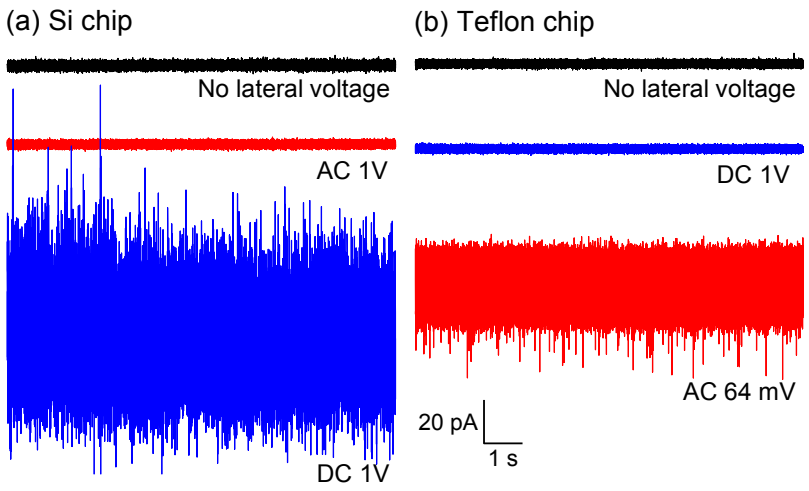

Fig. 4 Transmembrane current noise of BLM systems based on a Si chip (a) and a Teflon chip (b). Current noises without lateral voltage, with AC lateral voltage and DC lateral voltage were shown in black, red, and blue, respectively. The scale bar shown in (b) applies to all the figures. A low pass filter of 1 kHz was applied to all the recorded signals.

In the Teflon-chip-based system, it was observed that the BLM maintained a large resistance (>200 GΩ) and a small transmembrane current noise (~3 pA) when a DC lateral voltage was applied, as shown in Figure 4(b). However, when we applied an AC voltage to the Teflon chip, we observed that even a small voltage was sufficient to cause a drastic increase in noise level (>40 pA), and the AC voltage of 0.5 V (peak-to-peak) resulted in the rapture of the BLM. As shown above, the BLMs formed using the Teflon-chip and the Si-chip systems exhibited different sensitivities to noise when DC and AC lateral voltages were applied; to minimize transmembrane current noise induced by the application of the lateral voltage, DC voltages were preferable for BLMs in the Teflon chip systems, while AC voltages were preferable for BLMs in the case of Si chip systems.

Comparing these two systems, the pronounced differences lie in the flexibility of the membrane support (Teflon vs Si_3N_4), and the necessity of n-hexadecane to be present around the aperture. For the present, we tentatively assume that the BLMs formed in the flexible Teflon films are likely to vibrate mechanically due to the Coulomb force

from the AC lateral voltage between the two electrodes.[26,27] It is assumed that the mechanical vibration of the BLM would generate a displacement current near the BLM surface, which might result in the higher thermal noise in the transmembrane current in the case of the Teflon chip. On the other hand, we considered that the noise was suppressed in the Si chip due to its mechanical stability. At the same time, because it is necessary to apply n-hexadecane near the aperture to facilitate BLMs formation on Teflon chips, the edge of the lateral electrodes around the aperture would be in direct contact with the BLM. A DC lateral voltage would not induce an overly large electric field around the edge of the lateral electrodes. On the other hand, n-hexadecane is not required to form BLMs on Si chips. At the edge of the Si chip aperture, the BLM is in direct contact with the SiO_2-protected electrodes. Therefore, we speculate that when the lateral DC voltage is applied to the Si chip, a larger electric field would be generated at the sharp edges of the lateral electrodes, resulting in a large increase in noise and/or the electric breakdown of the insulating layer.

Effect of lateral voltage on fullerene-derivative-doped lipid bilayer membranes

BLMs formed across the tapered apertures in the Si chips were sufficiently stable to withstand high transmembrane voltages, which is suitable for sensor applications.[10] In a previous study, we introduced a fullerene derivative, phenyl-C_{61}-butyric acid methyl ester (PCBM), into BLMs, and observed that the lipid/PCBM hybrid membrane showed a reversible and repeatable response to light illumination.[17] However, the amplitude of the photoresponse was quite small, only about 20 pA. To increase the photo-response of the hybrid BLM, we used the above-mentioned electrode-equipped Si chip platform to introduce a lateral voltage to the lipid/PCBM hybrid BLM device.

The photoresponse from pure BLMs and PCBM doped BLMs under different lateral voltages are shown in Figure 5(a,b). In this experiment, relatively high transmembrane voltage of -1 V was used to effectively extract photo-generated electrons from the PCBM doped BLM. To minimize transmembrane current noise, an AC lateral voltage was applied to the electrodes of the Si chip. The pure BLM exhibited no detectable

response to light illumination or lateral voltage. The BLM doped with PCBM showed a photoresponse of about 22 pA at the lateral voltage of 0 V. This result is consistent with findings of our previous report.[17] As the lateral voltage was gradually increased, the amplitude of the photoresponse increased significantly (Figure 5(b)). As shown in Figure 5(c), when the lateral voltage was increased from 0 V to 3 V, the photoresponse increased by over 10 times (from 22 pA to 230 pA), indicating that the photoresponse can be effectively modulated and amplified by applying a lateral voltage to the BLM system. Compared with our previously reported results, both the amplitude and speed of the photoresponse increased.[18] These improvements can be attributed to two changes. First, we used a higher concentration of PCBM in the lipid solution (from 1 mg/mL to 2 mg/mL) to form the hybrid BLMs. This increased level of PCBM in the BLM would not only absorb more photons to generate a higher current, but also reduce the transmembrane resistance of the hybrid BLM, thereby increasing the response speed at the same time. Second, we used a green LED (525 nm) rather than an infrared LED (850 nm) to excite the PCBM in the BLM. Since PCBM has higher absorption coefficient at 525 nm than that at 850 nm, PCBM molecules in the present BLMs would absorb more photons which would also lead to a higher photoresponse.[28,29] These results fully demonstrates that the lateral voltage has a modulating effect on devices based on PCBM-doped BLMs.

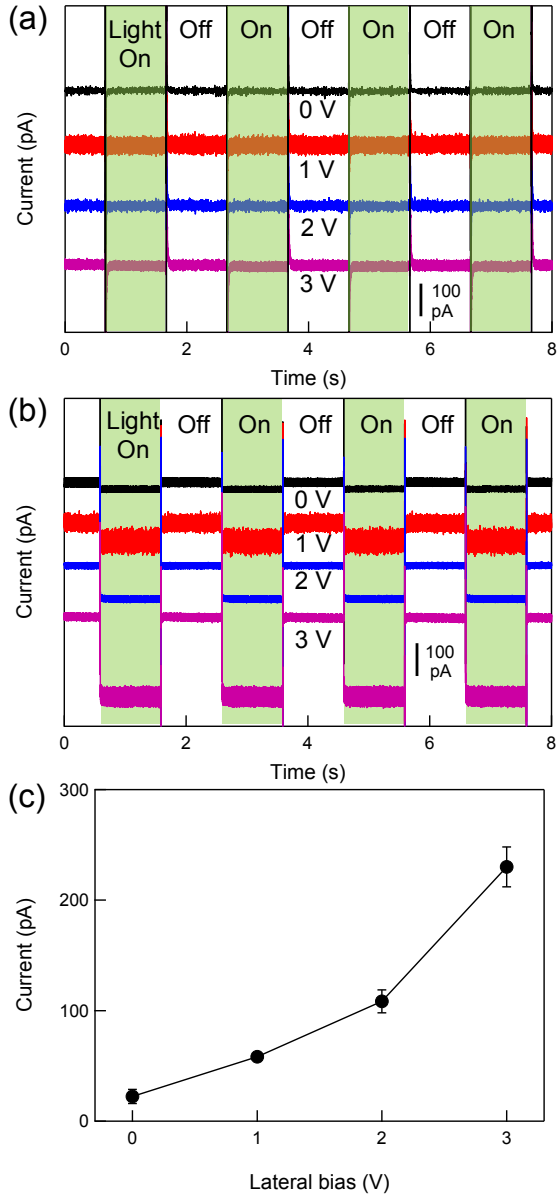

Fig. 5 Photoresponse from BLMs composed of pure DPhPC lipids (a) and PCBM-doped BLMs (b). (c) shows relationships between the photoresponse and applied lateral voltage. The statistical data are calculated based on the results of 3 separate experiments. The photoresponses were offset for clarity.

Effect of lateral voltage on lipid bilayer membranes with incorporated ion channels

Artificial BLMs have been used as an excellent platform for the functional analysis of biological ion channels.[2,4,19] We previously reported on successful recordings of

channel activities of various ion channels using BLM systems under the control of a transmembrane voltage.[9–12,24] However, we noticed that some biological channels rapidly lost their channel conducting activities, and once they went into a non-conductive state, they never returned to the conducting states that show channel opening and closing events. If another input, lateral voltage, was to be added to the BLM system, the lateral voltage might trigger channel conducting activities. We examined this possibility by using human $Na_V1.5$ channels as a representative example. The $Na_V1.5$ channel is a cardiac voltage-gated sodium channel that plays an essential role in initiating an action potential in the heart.[30,31]

BLMs were formed in the electrode-equipped Teflon chips and fused with proteoliposomes prepared from $Na_V1.5$-transfected HEK293T cells. On applying a voltage protocol (Figure 6(a)) that is commonly used for $Na_V1.5$ activation in patch-clamp studies[32,33], rectangular and spiky currents were sporadically observed (Figure 6(b)). However, after the repetition of the voltage sweeps, channel activities disappeared, and no channel activities were observed for more than 150 subsequent sweeps. Under the control of transmembrane voltage only, it was difficult to recover the channel activities from such a non-conducting state. When we applied a DC lateral voltage of 0.5 V to the BLM, the channel activities were dramatically enhanced, exhibiting consecutive opening and closing events. Because we recorded the channel currents at least 30 s after the application of the DC lateral voltage and the enhanced channel activities were observed over a relatively long period of time (>15 min), a steady-state process rather than a transient process (e.g. charging process of the lateral electrodes) is likely responsible for the enhanced channel activities. The enlarged view of the channel activities with lateral voltage was similar to that of the channel activities before entering non-conductive states (Figure 6(c)). The channel activities were blocked by the addition of tetrodotoxin (TTX), a specific blocker of sodium channels.[34,35] When we applied a lateral voltage to a BLM containing no ion channels, the same transmembrane voltage protocol induced no significant currents even when a higher lateral voltage (4 V) was applied (Figure 6(d)). These results indicate that the

application of the lateral voltages effectively recovered the activities of the NaV1.5 channel that were in non-conductive states. We therefore conclude that the lateral voltage represents an additional input in analyzing ion channel functions, which would have been hidden under the control of conventional transmembrane potentials.

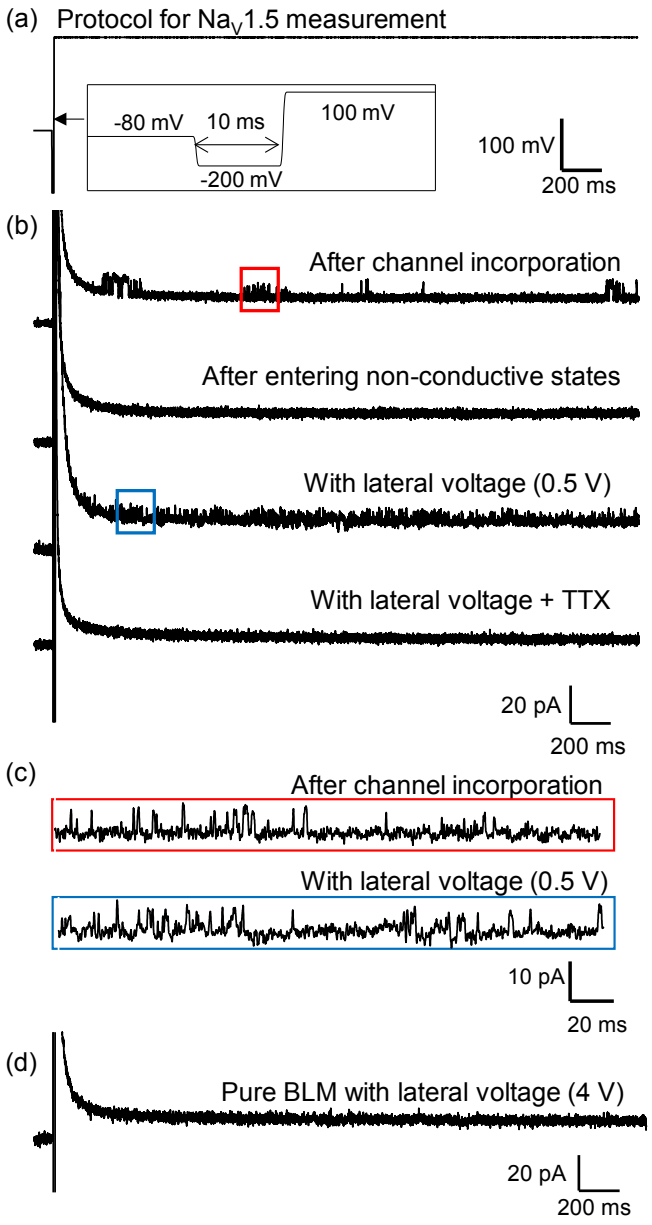

Fig. 6 (a) Transmembrane voltage protocol used for measuring $Na_V1.5$ channel currents. The inset shows the detail of the protocol. (b) Sequentially measured transmembrane currents using the voltage protocol: after the channel incorporation, after the channels lost their conductive activities, when a lateral voltage (0.5 V) was applied, after adding TTX to the buffer solution.

The measured currents were offset for clarity. (c) Enlarged views of the channel activities indicated by red and blue squares in (b). (d) Transmembrane current of a pure BLM when a lateral voltage (4 V) was applied.

Conclusions

In summary, a lateral voltage was introduced to a free-standing BLM system, which usually uses the transmembrane voltage as the only input. Electrode-equipped Teflon chips and Si chips were developed for applying lateral voltages to the BLMs. We successfully demonstrated that a lateral voltage could function as a "gate bias", and regulate the photocurrent flowing through the PCBM-doped BLMs. It has also been demonstrated that the ion current through an ion channel protein in the BLMs could also be activated by the application of a lateral voltage. These results indicate that the use of lateral voltages to enhance transmembrane currents represents a general approach irrespective of the channel materials used in the BLMs. Although further studies will be needed to elucidate the underlying mechanism responsible for modulating transmembrane currents by lateral voltages, we conclude (based on the findings reported herein), that the use of a lateral voltage represents a feasible new input that will dramatically expand the applicability of BLM systems.

Author contributions

Author Contributions: Conceptualization, A.H.-I.; Data curation, T.M. and M.K.; Investigation, M.S., M.K., T.W., X.F. and R.M.; Methodology, T.M. and A.H.-I.; Project administration, A.H.-I.; Resources, K.K., M.K., D.T. and Y.T.; Supervision, A.H.-I.; Validation, A.H.-I.; Visualization, T.M. and M.K.; Writing-original draft, T.M.; Writing-review and editing, T.M., F.H. and A.H.-I.. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

There are no conflicts to declare.

Acknowledgements

The work was supported by the CREST program of the Japan Science and Technology Agency (JPMJCR14F3), JSPS KAKENHI (19H00846, 20K20550), and partially supported by Asahi Glass Foundation, WPI-AIMR Fusion Research, and the Nation-wide Cooperative Research Project Program of the Research Institute of Electrical Communication at Tohoku University. Some of the equipment used in this research was manufactured by a technical staff member Kento Abe, at the machine shop division of Fundamental Technology Center, Research Institute of Electrical Communication, Tohoku University.

Note and references

1 P. Mueller, D. O. Rudin, H. Ti Tien and W. C. Wescott, *Nature*, 1962, **194**, 979–980.
2 C. G. Siontorou, G. P. Nikoleli, D. P. Nikolelis and S. K. Karapetis, *Membranes*, 2017, **7**, 38.
3 M. Bally, K. Bailey, K. Sugihara, D. Grieshaber, J. Vörös and B. Städler, *Small*, 2010, **6**, 2481–2497.
4 H. Lee, Y. Lee, S. S. Oh and S. Q. Choi, *Small*, 2020, **16**, 2002541.
5 M. Zagnoni, *Lab Chip*, 2012, **12**, 1026.
6 M. Montal and P. Mueller, *Proc. Natl. Acad. Sci. U. S. A.*, 1972, **69**, 3561–3566.
7 K. Funakoshi, H. Suzuki and S. Takeuchi, *Anal. Chem.*, 2006, **78**, 8169–8174.
8 R. Syeda, M. A. Holden, W. L. Hwang and H. Bayley, *J. Am. Chem. Soc.*, 2008, **130**, 15543–15548.
9 M. Komiya, M. Kato, D. Tadaki, T. Ma, H. Yamamoto, R. Tero, Y. Tozawa, M. Niwano and A. Hirano-Iwata, *Chem. Rec.*, 2020, **20**, 730–742.
10 D. Tadaki, D. Yamaura, S. Araki, M. Yoshida, K. Arata, T. Ohori, K. I. Ishibashi, M. Kato, T. Ma, R. Miyata, Y. Tozawa, H. Yamamoto, M. Niwano and A. Hirano-Iwata, *Sci. Rep.*, 2017, **7**, 17736.
11 A. Hirano-Iwata, Y. Ishinari, M. Yoshida, S. Araki, D. Tadaki, R. Miyata, K. Ishibashi, H. Yamamoto, Y. Kimura and M. Niwano, *Biophys. J.*, 2016, **110**,

2207–2215.
12 A. Oshima, A. Hirano-Iwata, H. Mozumi, Y. Ishinari, Y. Kimura and M. Niwano, *Anal. Chem.*, 2013, **85**, 4363–4369.
13 R. H. Tunuguntla, R. Y. Henley, Y.-C. Yao, T. A. Pham, M. Wanunu and A. Noy, *Science*, 2017, **357**, 792–796.
14 P. Urban, S. R. Kirchner, C. Mühlbauer, T. Lohmüller and J. Feldmann, *Sci. Rep.*, 2016, **6**, 22686.
15 S. Niu and D. Mauzerall, *J. Am. Chem. Soc.*, 1996, **118**, 5791–5795.
16 K. C. Hwang and D. Mauzerall, *Nature*, 1993, **361**, 138–140.
17 K. Kanomata, T. Deguchi, T. Ma, T. Haseyama, M. Miura, D. Yamaura, D. Tadaki, M. Niwano, A. Hirano-Iwata and F. Hirose, *J. Electroanal. Chem.*, 2019, **832**, 55–58.
18 T. Ma, X. Feng, T. Ohori, R. Miyata, D. Tadaki, D. Yamaura, T. Deguchi, M. Komiya, K. Kanomata, F. Hirose, M. Niwano and A. Hirano-Iwata, *ACS Omega*, 2019, **4**, 18299–18303.
19 T. Ma, M. Sato, M. Komiya, X. Feng, D. Tadaki and A. Hirano-Iwata, *Chem. Lett.*, 2021, **50**, 418–425.
20 T. Osaki and S. Takeuchi, *Anal. Chem.*, 2017, **89**, 216–231.
21 R. Miyata, D. Tadaki, D. Yamaura, S. Araki, M. Sato, M. Komiya, T. Ma, H. Yamamoto, M. Niwano and A. Hirano-Iwata, *Micromachines*, 2021, **12**, 98.
22 M. Mayer, J. K. Kriebel, M. T. Tosteson and G. M. Whitesides, *Biophys. J.*, 2003, **85**, 2684–2695.
23 M. Kitta, H. Tanaka and T. Kawai, *Biosens. Bioelectron.*, 2009, **25**, 931–934.
24 D. Yamaura, D. Tadaki, S. Araki, M. Yoshida, K. Arata, T. Ohori, K. Ishibashi, M. Kato, T. Ma, R. Miyata, H. Yamamoto, R. Tero, M. Sakuraba, T. Ogino, M. Niwano and A. Hirano-Iwata, *Langmuir*, 2018, **34**, 5615–5622.
25 T. Gutsmann, T. Heimburg, U. Keyser, K. R. Mahendran and M. Winterhalter, *Nat. Protoc.*, 2015, **10**, 188–198.
26 B. Sajadi, F. Alijani, D. Davidovikj, J. (Hans) Goosen, P. G. Steeneken and F. van Keulen, *J. Appl. Phys.*, 2017, **122**, 234302.
27 X. Zhang, K. Makles, L. Colombier, D. Metten, H. Majjad, P. Verlot and S. Berciaud, *Nat. Commun.*, 2020, **11**, 5526.
28 J. Y. Kim, K. Lee, N. E. Coates, D. Moses, T.-Q. Nguyen, M. Dante and A. J. Heeger, *Science*, 2007, **317**, 222–225.
29 L.-L. Deng, S.-L. Xie, C. Yuan, R.-F. Liu, J. Feng, L.-C. Sun, X. Lu, S.-Y. Xie, R.-B. Huang and L.-S. Zheng, *Sol. Energy Mater. Sol. Cells*, 2013, **111**, 193–199.
30 A. S. Amin, A. Asghari-Roodsari and H. L. Tan, *Pflügers Arch. - Eur. J. Physiol.*, 2010, **460**, 223–237.
31 W. A. Catterall, *J. Physiol.*, 2012, **590**, 2577–2589.
32 G. Klein, A. Gardiwal, A. Schaefer, B. Panning and D. Breitmeier, *Forensic Sci. Int.*, 2007, **171**, 131–135.
33 A. Beyder, J. L. Rae, C. Bernard, P. R. Strege, F. Sachs and G. Farrugia, *J. Physiol.*, 2010, **588**, 4969–4985.

34 M. Stevens, S. Peigneur and J. Tytgat, *Front. Pharmacol.*, 2011, **2**, 71.
35 M. Kollarik, H. Sun, R. A. Herbstsomer, F. Ru, M. Kocmalova, S. N. Meeker and B. J. Undem, *J. Physiol.*, 2018, **596**, 1419–1432.