Kobe University Repository : Kernel

PDF issue: 2025-08-10

Substrate-supported Model Membrane as a Versatile Analytical/Biosensing Platform

Morigaki, Kenichi

(Citation)
Analytical Sciences,37(5):683-689

(Issue Date)
2021-05

(Resource Type)
journal article

(Version)
Version of Record

(Rights)
© 2021 by The Japan Society for Analytical Chemistry

(URL)
https://hdl.handle.net/20.500.14094/90008391

2021 © The Japan Society for Analytical Chemistry

Reviews

Substrate-supported Model Membrane as a Versatile Analytical/ Biosensing Platform

Kenichi MORIGAKI*,**

*Biosignal Research Center, Kobe University, 1-1 Rokkodaicho, Nada, Kobe 657–8501, Japan
**Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodaicho, Nada, Kobe 657–8501, Japan

Biological membranes composed of a lipid bilayer and associated proteins work as a platform for highly selective and sensitive detection in nature. Substrate-supported lipid bilayers (SLBs) are a model system of the biological membrane that are mechanically stable, accessible to highly sensitive analytical techniques, and amenable to micro-fabrication, such as patterning. The surface of SLBs can effectively suppress the non-specific binding of proteins, and enhance selective detection by specific interactions. These features render SLBs highly attractive for the development of devices that utilize artificially mimicked cellular functions. Furthermore, SLBs can be combined with nanoscopic spaces, such as nano-channels and nano-pores, that can reduce the detection volume and suppress the non-specific background noise, enhancing the signal-to-background noise (S/B) ratio. SLBs therefore provide promising platforms for a wide range of biomedical and environmental analyses.

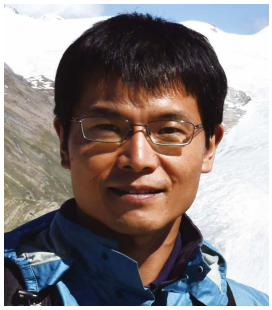
Keywords Biological membrane, model membrane, lipid bilayer, supported lipid bilayer, nanofluidics

(Received November 28, 2020; Accepted January 7, 2021; Advance Publication Released Online by J-STAGE January 15, 2021)

1 Introduction 683
2 Substrate-supported Lipid Bilayers as a Versatile Analytical Platform 683
3 Confinement of Lipid Membranes in a Nanoscopic Space 684
4 Nanogap-junction: A Novel Nanoscopic Analytical Platform 685
5 Conclusions and Perspectives 687
6 Acknowledgements 688
7 References 688

1 Introduction

The detection of specific molecules in solutions containing diverse coexisting molecules is important in a wide range of biomedical and environmental analyses. In nature, biological membranes composed of a lipid bilayer and associated proteins work as a platform for highly selective and sensitive detection, owing to their unique structural and physicochemical properties. Biological membranes involve two-dimensional fluid, in which membrane-bound molecules can diffuse laterally.[1–4] In addition, the phospholipid-based membrane surface can suppress the nonspecific adsorption of proteins, thus enhancing the specific binding by molecular recognition.[5] These features have provided unique advantages to detect specific target molecules and suppress nonspecific background noise from coexisting molecules.[6,7] From this aspect, biological membranes have been an important motif for developing materials for analytical techniques and sensors.



Kenichi MORIGAKI received his bachelo's and master's degree from the University of Tokyo, and Ph.D. from Swiss Federal Institute of Technology (ETH-Zurich, Switzerland). After working as a postdoctoral fellow at Max-Planck-Institute of Polymer Research (Mainz, Germany) and ETH-Zurich, he joined the National Institute of Advanced Industrial Science and Technology (AIST) in 2002. Since 2009, he is an associate professor at Kobe University. His research interest is oriented towards complex self-assembly of biological/environmental systems, especially biological membranes.

E-mail: morigaki@port.kobe-u.ac.jp

2 Substrate-supported Lipid Bilayers as a Versatile Analytical Platform

Synthetic model systems of the biological membranes, such as Langmuir monolayer, lipid vesicle, and black lipid membrane (BLM), have played important roles in understanding the structures and functions of the biological membranes.[8] They also provide platforms for biosensing and biomedical applications by mimicking the membrane functions. Substrate-supported lipid bilayers (SLBs), a relatively new breed of the model membranes introduced in 1980's,[9,10] typically comprise a single lipid bilayer adsorbed on a solid surface by physical

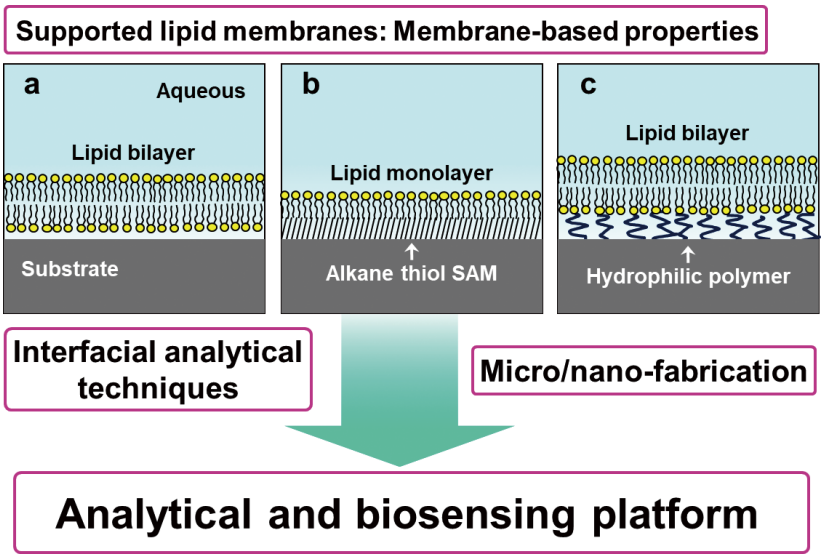


Fig. 1 Schematic drawing of the supported membranes formats: (a) Supported lipid bilayer (SLB), (b) hybrid bilayer of self-assembled monolayer and lipid monolayer, (c) tethered bilayer with a hydrophilic support to separate the membrane and the substrate.

Table 1 Important features and applied techniques of SLBs

Features	Applied techniques	References
Mechanical stability	Tethered bilayer	60, 61, 62, 63
	Polymerizable lipid bilayer	48, 65, 66, 67, 68, 69, 70
Analytical techniques	TIRF	11
	SPR	12, 13
	QCM-D	14, 15
	Electrical detection (EIS)	12, 16
Micro-pattering	Photolithography	16, 17, 18
	Soft lithography	19
Nanoscopic confinement	ZMW	45
	Nanopore	41, 42
	Nano-channel	46, 51, 52

interactions or chemical bonds (Fig. 1). SLBs have some unique features compared with other formats of model membranes, such as mechanical stability and accessibility to highly sensitive optical and electrochemical analytical techniques (*e.g.* total internal reflection fluorescence microscopy, surface plasmon resonance (SPR), quartz-crystal-micro-balance (QCM), and electrical impedance spectroscopy (EIS)) (Table 1).[11-16] SLBs can also be generated in micropatterns by applying lithographic techniques, thus allowing one to create designed arrays of model membranes.[17-20] Since the surface of SLBs is based on phospholipids that can effectively suppress non-specific binding of proteins, they can enhance the selective detection of biological molecules by specific interactions.[5] Furthermore, an important feature of SLB is its fluidity, *i.e.* the fact that lipid bilayers are in a liquid-crystalline state, and embedded molecules can laterally diffuse.[21,22] These features render SLBs highly attractive for the development of devices that utilize artificially mimicked cellular functions. The detection of biological/environmental molecules using SLB platforms has been extensively studied. For example, interactions between small molecules (*e.g.* drugs) and lipid membranes have been studied using SLBs.[23,24] Two-dimensionally fluid SLBs were used for immunoassays by attaching ligands or receptors to the membrane surface.[16,25] Cornell *et al.* have developed a biosensing technique that transduces the molecular-recognition event to a conductance change by utilizing membrane-embedded ion channels. By linking a gramicidin ion channel to antibody fragments (Fab'), the binding of analyte molecules to Fab' was detected as conductance changes due to the modulation of gramicidin dimerization.

3 Confinement of Lipid Membranes in a Nanoscopic Space

Many analytical techniques employ a smaller detection volume in the micrometer and nanometer range to enhance the sensitivity by suppressing the background noise and increasing the signal-to-background noise ratio (S/B ratio).[26] For example, total-internal-reflection fluorescence microscopy (TIR-FM) has enabled to detect single biological molecules (*e.g.* motor proteins) and provided with detailed information on their molecular machinery.[27-29] More recently, micro-fabrication techniques have been applied to create yet smaller spaces, including zero-mode-waveguide (ZMW), [30,31] nanofluidics, [32-34] micro-chambers, [35-38] and nanopores.[39-42] ZMW and nanopores are already being applied to DNA- and RNA-sequencing.[31,43] They can provide fluorescence or electrical signals for each base as a long continuous DNA chain goes through the nanoscopic space. The determination of sequences based on single molecule detection resulted in unique advantages, such as high efficiency by the parallel reading and long read of the sequences.

Several lines of research have been pursued to combine SLBs with a nanometric space in order to realize synergetic effects of highly sensitive detection tools and intrinsically selective/sensitive biological membrane architecture (Fig. 2). For example, TIR-FM observation of SLB has been exploited in the analytical applications of biological molecules.[11,44] Nano-fabricated platforms, such as ZMW and nanopores, have also

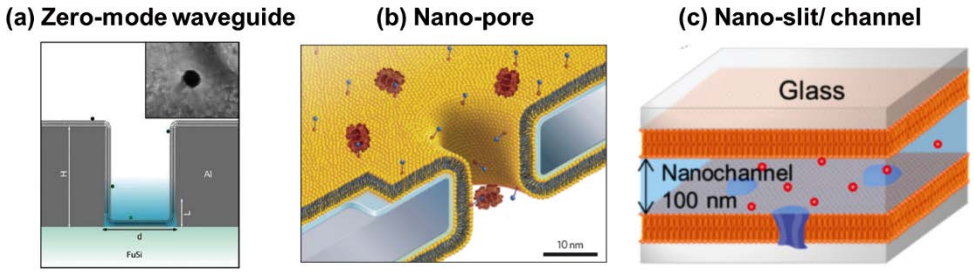


Fig. 2 Nanofabricated structures that can generate a nanoscopic space: (a) Zero-mode-waveguide (ZMW), (b) Nano-pore, (c) Nano-channel (nano-slit). The images were adapted from Samiee *et al.*, 2006, Yusko *et al.*, 2011, and Kazoe *et al.*, 2020.

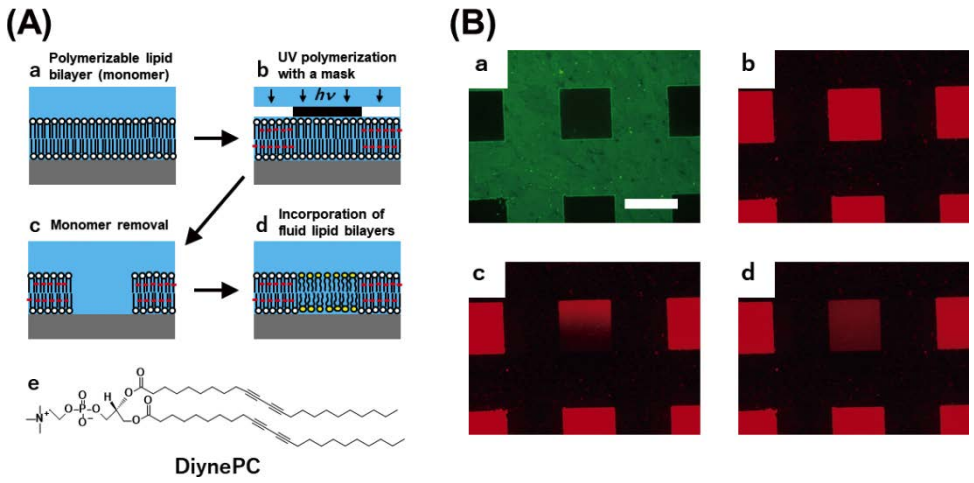


Fig. 3 (A) Schematic outline of the patterning procedure and the structure of diacetylene phospholipid, DiynePC. (B) Fluorescence micrographs of a patterned bilayer. (a) Green fluorescence from the bilayer of polymerized DiynePC. (b) Red fluorescence from fluid bilayers (phosphatidylcholine from egg yolk (egg-PC)/1 mol% Texas Red 1,2-dihexadecanoyl-*sn*-glycero-phosphoethanolamine (TR-PE)) in the wells between polymerized bilayers (corrals). (c) – (d) Local photobleaching of TR-PE resulted in a homogeneously dark corral, demonstrating the lateral diffusion of lipid molecules. The scale bar corresponds to 50 µm. The images were adapted from Morigaki *et al.*, 2004.

been utilized in combination with SLBs, yielding distinctive advantages of nanoscopic spaces.[45] Yusko *et al.* have shown that coating nanopores with a fluid lipid bilayer could suppress nonspecific binding and aggregation of proteins on the surface, and improved the electrical detection of single molecules passing through the pore by slowing down the translocation of target proteins.[41] The same research group has utilized lipid-coated nanopores to determine the shape, volume, charge, rotational diffusion coefficient and dipole moment of individual proteins in the pore, enabling to identify, characterize and quantify proteins and protein complexes.[42] By working with a lipid-coated glass nanochannel, Kazoe *et al.* studied the physicochemical properties of water in confined spaces.[46] They revealed that the viscosity of water in a nanochannel with the thickness of 200 nm was 2.1 – 5.6 times higher than the bulk water phase, suggesting that interactions between water molecules and a lipid bilayer surface significantly affect the molecular/ion transport. The slowed diffusion in a lipid-coated nanoscopic space also has important implications towards the behaviors of biological molecules in the cellular interior and cell-cell junction. Nanofluidic systems are currently being explored for novel functionalities and applications owing to its unique properties that are not observed in microscale or bulk solutions.[47] By combining these features with the biomimetic characteristics of SLBs, these platforms have promising potentials for the analysis of biological molecules.

4 Nanogap-junction: A Novel Nanoscopic Analytical Platform

As a model membrane that can reproduce the lateral mobility and compartmentalization of a biological membrane in a technologically amenable format, we developed a micropatterning strategy of SLBs by applying the photo-lithographic polymerization of lipid bilayers.[48-50] An SLB of polymerizable diacetylene-containing phospholipid (DiynePC) was first formed on a hydrophilic substrate, such as glass, and photo-polymerized by UV irradiation (Fig. 3). Subsequently, monomeric DiynePC was selectively removed with a detergent solution. Thus formed patterned polymeric SLB was used as a framework to incorporate natural phospholipid bilayers, forming a hybrid membrane of two distinctive lipid bilayer regions. The polymerized bilayer (hereafter called "polymeric bilayer")

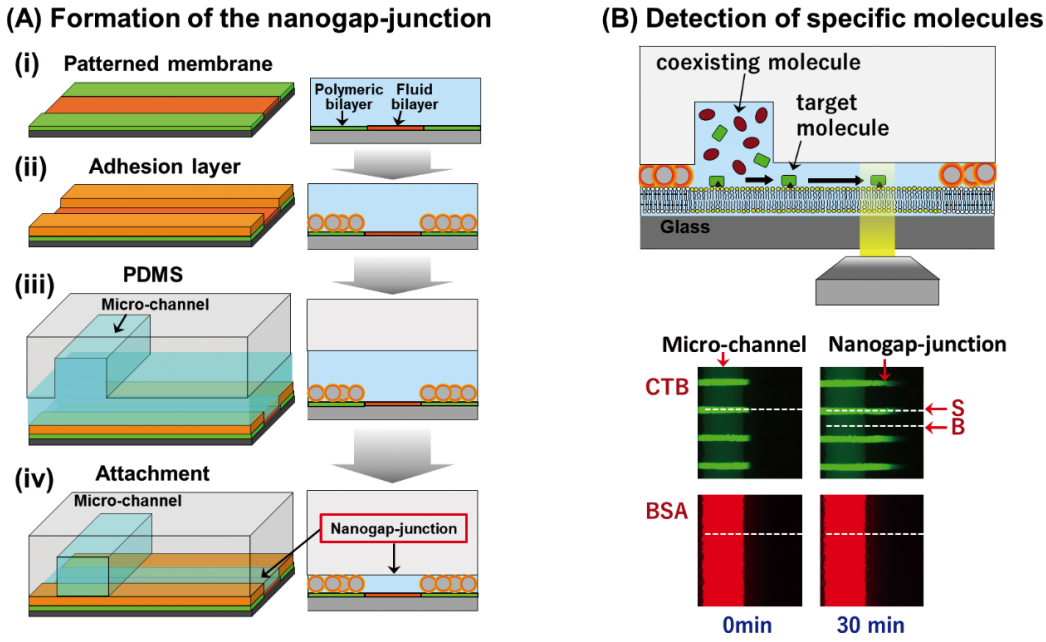


Fig. 4 Schematic illustration of the nanogap-junction. (A) Formation process: the polymeric bilayer and PDMS are attached with an adhesion layer, forming a gap structure between the fluid bilayer and PDMS (nanogap-junction). (B) Detection of specific target molecules: from a mixture of target and coexisting molecules in the microchannel, target molecules are selectively transported into the nanogap junction by specific binding and lateral diffusion on the fluid bilayer. The fluorescence micrographs show the selective penetration of target proteins into nanogap-junctions. A mixed solution of target protein (CTB) and coexisting protein (BSA) was introduced into a microchannel. CTB could bind onto the fluid bilayer and migrated into the nanogap junctions with time (green channel), whereas BSA remained in the microchannel (red channel). The signs S and B represent the regions where the signal and background fluorescence were measured, respectively. The images were adapted from Tanabe *et al.*, 2018 and Ando *et al.*, 2016.

provides mechanical stability, whereas the natural phospholipid bilayer (hereafter called "fluid bilayer") possesses the lateral mobility of molecules (fluidity) and biomimetic functionality (*e.g.* molecular recognition by surface receptors). In order to realize a highly sensitive analytical platform based on the two-dimensional (2D) configuration of a patterned membrane, it was combined with a nanometric detection volume by forming a very thin aqueous space between the fluid bilayer and a silicone elastomer (polydimethylsiloxane: PDMS) sheet (nanogap-junction) (Fig. 4). To this end, a PDMS sheet was attached to the surface of a polymeric bilayer using an adhesion material (glue) with a pre-defined thickness (*e.g.* lipid vesicles, silica nanoparticles, and hydrophilic polymer brushes). The fabrication process of a nanogap-junction is outlined in Fig. 4(A). The adhesion material (*e.g.* silica nanoparticles) was attached onto the surface of the polymeric bilayer by using the biotin-streptavidin linkage (both the polymeric bilayer and the adhesion material had a biotin moiety, and streptavidin was used to link them). In the same way, PDMS having the biotin-modified surface was attached onto the surface of the adhesion layer. The thickness of the nanogap-junction was determined by the adhesion layer, and we could generate a gap thickness of less than 100 nm.[51] The combination of a nanometric gap structure and a fluid lipid bilayer enabled to selectively transport and detect target molecules that could bind to the membrane surface by specific molecular recognition, whereas the nonspecific penetration of coexisting molecules was suppressed. In Fig. 4, the model target molecule (cholera toxin subunit B: CTB) could bind to a fluid bilayer containing its ligand (G_{M1}) and penetrate into the nanogap-junction by lateral diffusion (green fluorescence). In contrast, the model coexisting molecule (bovine serum albumin: BSA) could not bind to the membrane surface, and mostly remained in the solution in the microchannel. The selective transport of CTB into the nanogap-junction contributed to enhance the S/B ratio (*i.e.* the ratio of fluorescence intensities from specificcally bound CTB and the non-specific background noise measured in the polymeric bilayer regions, as indicated with signs S and B in Fig. 4). The use of silica nanoparticles as the adhesion layer enabled to control the thickness of the nanogap-junction accurately due to the uniform sizes of silica nanoparticles.[52] By using silica nanoparticles having a smaller size, we could generate a thinner nanogap-junction, which realized a higher S/B ratio (Fig. 5). In the micro-channel, the background fluorescence of BSA was very intense, and the fluorescence from membrane-bound CTB in the fluid bilayer was hardly observable (Fig. 5A). The signal from CTB was more clearly visible in the nanogap-junction due to the suppressed background fluorescence, because BSA molecules were mostly excluded. The levels of the signal and background are shown with pink and blue lines in Fig. 5A, respectively. The background fluorescence intensity was more effectively suppressed for 30 nm nanoparticles compared with 100 nm nanoparticles, because a thinner gap is more effective in eliminating BSA, thus enhancing the S/B ratio even more drastically (Fig. 5B). Furthermore, silica nanoparticles are mechanically robust, and the nanogap-junctions formed had a long life time (> week). The stability allows one to store a pre-assembled biochip having a nanogap-junction, thus

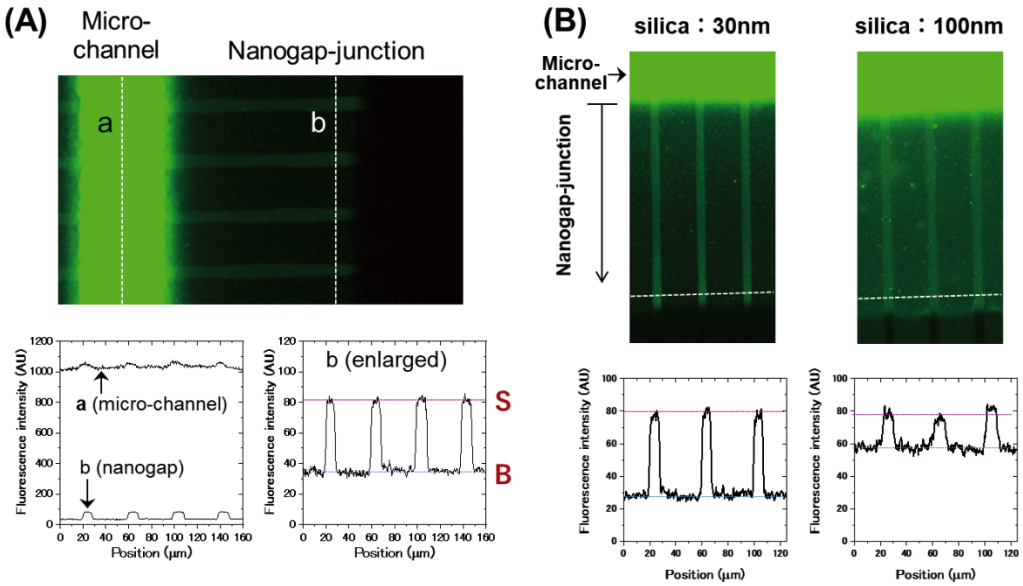


Fig. 5 Selective transport of target molecules into the nanogap-junction formed with silica nanoparticles: (A) Mixed solution of CTB (5 μg/mL) and BSA-488 (200 μg/mL) (both emitting green fluorescence) was introduced into the micro-channel. In the micro-channel, the background fluorescence of BSA was much more intense than the fluorescence from CTB bound on the fluid bilayer. The signal from CTB was more clearly visible in the nanogap-junction due to suppressed background fluorescence from BSA. The pink line with the sign S and the blue line with the sign B represent the levels of the signal and background, respectively. (B) Effect of silica nanoparticle sizes on the suppression of background noise: fluorescence arising from a mixture of BSA and CTB was compared in the nanogap-junction formed with 30 and 100 nm silica nanoparticles. The background fluorescence intensity was more effectively suppressed for 30 nm nanoparticles compared with 100 nm nanoparticles, thus enhancing the S/B ratio. The images were adapted from Tanabe *et al.*, 2018.

eliminating the necessity for elaborate onsite assembly process. Compared to other techniques used for ultrasensitive detection, the nanogap-junction is unique in the following points. First, unlike TIRF, one does not need the total internal reflection of the incident light, significantly simplifing the illumination setup and enabling the use of cost-effective light sources, such as LEDs. Second, unlike ZMW, which measures single molecules at the bottom of nanoscopic wells, a nanogap-junction offers an open two-dimensional space where the lateral movement of single molecules can be tracked. It enables to monitor the lateral positions, movement, and binding/unbinding of membrane-bound molecules in real time. These features should provide a uniquely versatile platform for biosensing and analytical applications.

5 Conclusions and Perspectives

The lateral and vertical organizations are the ubiquitous hallmark of biological membranes in living systems, and they enable a highly sensitive detection of specific target molecules. SLB can, at least partially, reproduce these structural features of a biological membrane, together with physicochemical properties, such as lateral fluidity and resistance towards nonspecific adsorption. Furthermore, SLB is amenable to interfacial analytical techniques and microfabrication technology to enhance the sensitivity and specificity of detection. The combination of SLB and nanoscopic space has been exploited in various formats, including ZMW, nanopores, and nanochannels. We recently developed a nanometric gap structure, called the nanogap-junction, to selectively transport and detect specific target molecules, while suppressing the penetration of non-target molecules. These formats could possibly be applied to a wide range of biomedical and environmental analyses by detecting, for example, biomarker molecules using membrane-bound receptors and antibodies.

There are several important challenges for the development of membrane-based analyses. First, the majority of receptor molecules in a biological membrane are integral membrane proteins, such as G-protein coupled receptors (GPCRs). The reconstitution of membrane proteins into an SLB is therefore very important. However, it poses significant technological challenges. Currently, membrane proteins are reconstituted either *via* the detergent-solubilized states or directly using cell-derived membranes.[53–56] However, the efficiency of reconstitution is still rather low. Various new approaches are currently under way, including the use of new detergents and nanodisks.[57,58] Furthermore, the physicochemical properties of reconstituted proteins, such as the lateral mobility and functionality, are often hampered due to the proximity of the substrate surface.[59] Model membranes with a controlled distance from the substrate have been developed using hydrophilic spacer materials such as a polymer cushion [60-63] and a micro-structured substrate.[64]

Another critical issue is the stability of lipid membranes. Lipid bilayer structures are generally formed by weak physical interactions, such as hydrophobic, electrostatic, and steric interactions, and they are susceptible to destabilization by perturbations such as air bubbles and amphiphiles. Improving the stability of lipid membranes is very important for the practical applications. Polymerization of lipids in bilayers has been extensively studied for stabilizing lipid vesicles (liposomes)

with encapsulated medicinal materials in drug delivery applications.[65] Upon polymerization, the bilayer structure became significantly more stable.[66,67] Polymerized SLBs have shown enhanced durability in such applications as capillary electrophoresis and single ion channel detection.[68,69] Furthermore, completely synthetic polymer-based bilayer membranes have been devised to mimic the membrane properties and functions, while also realizing the reconstitution of membrane proteins.[70] Such natural/artificial hybrid membranes would hold promise to extend the territories of membrane-based analytical techniques.

6 Acknowledgements

The development of "nanogap-junction" was partially supported by a Grant-in-Aid for Scientific Research on Innovative Areas "Molecular Soft-Interface Science". We would like to express gratitude to Dr. Mizuo Maeda for the fruitful project of "Molecular Soft-Interface Science", which was critical in facilitating the development.

7 References

1. S. J. Singer and G. L. Nicolson, *Science*, **1972**, *175*, 720.
2. K. Simons and D. Toomre, *Nature Rev. Mol. Cell Biol.*, **2000**, *1*, 31.
3. G. Vereb, J. Szöllosi, J. Matkó, P. Nagy, T. Farkas, L. Vigh, L. Mátyus, T. A. Waldmann, and S. Damjanovich, *Proc. Natl. Acad. Sci. U. S. A.*, **2003**, *100*, 8053.
4. A. Kusumi, T. K. Fujiwara, R. Chadda, M. Xie, T. A. Tsunoyama, Z. Kalay, R. S. Kasai, and K. G. N. Suzuki, *Annu. Rev. Cell Dev. Biol.*, **2012**, *28*, 215.
5. D. Chapman, *Langmuir*, **1993**, *9*, 39.
6. D. J. Irvine, M. A. Purbhoo, M. Krogsgaard, and M. M. Davis, *Nature*, **2002**, *419*, 845.
7. T. Doan, A. Mendez, P. B. Detwiler, J. Chen, and F. Rieke, *Science*, **2006**, *313*, 530.
8. M. Edidin, *Nature Rev. Mol. Cell Biol.*, **2003**, *4*, 414.
9. A. A. Brian and H. M. McConnell, *Proc. Natl. Acad. Sci. U. S. A.*, **1984**, *81*, 6159.
10. L. K. Tamm and H. M. McConnell, *Biophys. J.*, **1985**, *47*, 105.
11. E. Kalb, S. Frey, and L. K. Tamm, *Biochim. Biophys. Acta*, **1992**, *1103*, 307.
12. S. Lingler, I. Rubinstein, W. Knoll, and A. Offenhäusser, *Langmuir*, **1997**, *13*, 7085.
13. K. Tawa and K. Morigaki, *Biophys. J.*, **2005**, *89*, 2750.
14. C. A. Keller and B. Kasemo, *Biophys. J.*, **1998**, *75*, 1397.
15. R. P. Richter, R. Berat, and A. R. Brisson, *Langmuir*, **2006**, *22*, 3497.
16. B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek, and R. J. Pace, *Nature*, **1997**, *387*, 580.
17. J. T. Groves, N. Ulman, and S. G. Boxer, *Science*, **1997**, *275*, 651.
18. J. T. Groves and S. G. Boxer, *Acc. Chem Res.*, **2002**, *35*, 149.
19. C. K. Yee, M. L. Amweg, and A. N. Parikh, *Adv. Mater.*, **2004**, *16*, 1184.
20. S. Majd and M. Mayer, *Angew. Chem. Int. Ed.*, **2005**, *44*, 6697.
21. J. T. Groves, S. G. Boxer, and H. M. McConnell, *Proc. Natl. Acad. Sci. U. S. A.*, **1997**, *94*, 13390.
22. M. R. Cheetham, J. P. Bramble, D. G. G. McMillan, R. J. Bushby, P. D. Olmsted, L. J. C. Jeuken, and S. D. Evans, *Soft Matter*, **2012**, *8*, 5459.
23. T. T. Nguyen and J. C. Conboy, *Anal. Chem.*, **2011**, *83*, 5979.
24. D. Huang, T. Zhao, W. Xu, T. Yang, and P. S. Cremer, *Anal. Chem.*, **2013**, *85*, 10240.
25. T. Yang, S. Jung, H. Mao, and P. S. Cremer, *Anal. Chem.*, **2001**, *73*, 165.
26. J. J. Gooding and K. Gaus, *Angew. Chem. Int. Ed.*, **2016**, *55*, 11354.
27. D. Axelrod, *J. Cell Biol.*, **1981**, *89*, 141.
28. T. Funatsu, Y. Harada, M. Tokunaga, K. Saito, and T. Yanagida, *Nature*, **1995**, *374*, 555.
29. H. Noji, R. Yasuda, M. Yoshida, and K. Kinoshita Jr., *Nature*, **1997**, *386*, 299.
30. M. J. Levene, J. Korlach, S. W. Turner, M. Foquet, H. G. Craighead, and W. W. Webb, *Science*, **2003**, *299*, 682.
31. J. Eid, A. Fehr, J. Gray, K. Luong, J. Lyle, G. Otto, P. Peluso, D. Rank, P. Baybayan, B. Bettman, A. Bibillo, K. Bjornson, B. Chaudhuri, F. Christians, R. Cicero, S. Clark, R. Dalal, A. deWinter, J. Dixon, M. Foquet, A. Gaertner, P. Hardenbol, Cheryl Heiner, K. Hester, D. Holden, G. Kearns, X. Kong, R. Kuse, Y. Lacroix, S. Lin, P. Lundquist, C. Ma, P. Marks, M. Maxham, D. Murphy, I. Park, T. Pham, M. Phillips, J. Roy, R. Sebra, G. Shen, Jon Sorenson, A. Tomaney, K. Travers, M. Trulson, J. Vieceli, J. Wegener, D. Wu, A. Yang, D. Zaccarin, P. Zhao, F. Zhong, J. Korlach, and S. Turner, *Science*, **2009**, *323*, 133.
32. M. Krishnan, I. Mönch, and P. Schwille, *Nano Lett.*, **2007**, *7*, 1270.
33. P. Abgrall and N. T. Nguyen, *Anal. Chem.*, **2008**, *80*, 2326.
34. K. Mawatari, Y. Kazoe, H. Shimizu, Y. Pihosh, and T. Kitamori, *Anal. Chem.*, **2014**, *86*, 4068.
35. T. A. Dickinson, J. White, J. S. Kauer, and D. R. Walt, *Nature*, **1996**, *382*, 697.
36. Y. Rondelez, G. Tresset, K. V. Tabata, H. Arata, H. Fujita, S. Takeuchi, and H. Noji, *Nat. Biotechnol.*, **2005**, *23*, 361.
37. D. M. Rissin, C. W. Kan, T. G. Campbell, S. C. Howes, D. R. Fournier, L. Song, T. Piech, P. P. Patel, L. Chang, A. J. Rivnak, E. P. Ferrell, J. D. Randall, G. K. Provuncher, D. R. Walt, and D. C. Duffy, *Nat. Biotechnol.*, **2010**, *28*, 595.
38. S. H. Kim, S. Iwai, S. Araki, S. Sakakihara, R. Iino, and H. Noji, *Lab Chip*, **2012**, *12*, 4986.
39. J. J. Kasianowicz, E. Brandin, D. Branton, and D. W. Deamer, *Proc. Natl. Acad. Sci. U. S. A.*, **1996**, *93*, 13770.
40. D. Branton, D. W. Deamer, A. Marziali, H. Bayley, S. A. Benner, T. Butler, M. D. Ventra, S. Garaj, A. Hibbs, X. Huang, S. B. Jovanovich, P. S. Krstic, S. Lindsay, X. S. Ling, C. H. Mastrangelo, A. Meller, J. S. Oliver, Y. V. Pershin, J. M. Ramsey, R. Riehn, G. V. Soni, V. Tabard-Cossa, M. Wanunu, M. Wiggin, and J. A. Schloss, *Nat. Biotechnol.*, **2008**, *26*, 1146.
41. E. C. Yusko, J. M. Johnson, S. Majd, P. Prangkio, R. C. Rollings, J. Li, J. Yang, and M. Mayer, *Nat. Nanotechnol.*, **2011**, *6*, 253.
42. E. C. Yusko, B. R. Bruhn, O. M. Eggenberger, J. Houghtaling, R. C. Rollings, N. C. Walsh, S. Nandivada, M. Pindrus, A. R. Hall, D. Sept, J. Li, D. S. Kalonia, and M. Mayer, *Nat. Nanotechnol.*, **2017**, *12*, 360.
43. S. Howorka, *Nat. Nanotechnol.*, **2017**, *12*, 619.
44. D. W. Lee, H.-L. Hsu, K. B. Bacon, and S. Daniel, *PLoS One*, **2016**, *11*, e0163437.
45. K. T. Samiee, J. M. Moran-Mirabal, Y. K. Cheung, and H. G. Craighead, *Biophys. J.*, **2006**, *90*, 3288.

46. Y. Kazoe, K. Mawatari, L. Li, H. Emon, N. Miyawaki, H. Chinen, K. Morikawa, A. Yoshizaki, P. S. Dittrich, and T. Kitamori, *J. Phys. Chem. Lett.*, **2020**, *11*, 5756.
47. T. H. H. Le, H. Shimizu, and K. Morikawa, *Micromachines*, **2020**, *11*, 885.
48. K. Morigaki, T. Baumgart, A. Offenhäusser, and W. Knoll, *Angew. Chem. Int. Ed.*, **2001**, *40*, 172.
49. K. Morigaki, K. Kiyosue, and T. Taguchi, *Langmuir*, **2004**, *20*, 7729.
50. K. Morigaki, K. Mizutani, M. Saito, T. Okazaki, Y. Nakajima, Y. Tatsu, and H. Imaishi, *Langmuir*, **2013**, *29*, 2722.
51. K. Ando, M. Tanabe, and K. Morigaki, *Langmuir*, **2016**, *32*, 7958.
52. M. Tanabe, K. Ando, R. Komatsu, and K. Morigaki, *Small*, **2018**, *14*, 1802804.
53. V. Subramaniam, G. D. D'Ambruoso, J. H. K. Hall, J. Ronald J. Wysocki, M. F. Brown, and S. S. Saavedra, *Langmuir*, **2008**, *24*, 11067.
54. A. Sumino, T. Dewa, T. Noji, Y. Nakano, N. Watanabe, R. Hildner, N. Bösch, J. r. Köhler, and M. Nango, *J. Phys. Chem. B*, **2013**, *117*, 10395.
55. Y. Tanimoto, K. Okada, F. Hayashi, and K. Morigaki, *Biophys. J.*, **2015**, *109*, 2307.
56. M. J. Richards, C.-Y. Hsia, R. R. Singh, H. Haider, J. Kumpf, T. Kawate, and S. Daniel, *Langmuir*, **2016**, *32*, 2963.
57. A. Urbani, V. Giorgio, A. Carrer, C. Franchin, G. Arrigoni, C. Jiko, K. Abe, S. Maeda, K. Shinzawa-Itoh, J. F. M. Bogers, D. G. G. McMillan, C. Gerle, I. Szabò, and P. Bernardi, *Nat. Commun.*, **2019**, *10*, 4341.
58. A. Luchini, F. G. Tidemand, N. T. Johansen, M. Campana, J. Sotres, M. Ploug, M. Cárdenas, and L. Arleth, *Anal. Chem.*, **2019**, *92*, 1081.
59. A. Graneli, J. Rydstrom, B. Kasemo, and F. Höök, *Langmuir*, **2003**, *19*, 842.
60. M. Tanaka and E. Sackmann, *Nature*, **2005**, *437*, 656.
61. M. A. Deverall, E. Gindl, E. K. Sinner, H. Besir, J. Ruehe, M. J. Saxton, and C. A. Naumann, *Biophys. J.*, **2005**, *88*, 1875.
62. E. A. Smith, J. W. Coym, S. M. Cowell, T. Tokimoto, V. J. Hruby, H. I. Yamamura, and M. J. Wirth, *Langmuir*, **2005**, *21*, 9644.
63. T. Nishimura, F. Tamura, S. Kobayashi, Y. Tanimoto, F. Hayashi, Y. Sudo, Y. Iwasaki, and K. Morigaki, *Langmuir*, **2017**, *33*, 5752.
64. R. Watanabe, N. Soga, D. Fujita, K. V. Tabata, L. Yamauchi, S. H. Kim, D. Asanuma, M. Kamiya, Y. Urano, H. Suga, and H. Noji, *Nat. Commun.*, **2014**, *5*, 4519.
65. A. Mueller and D. F. O'Brien, *Chem. Rev.*, **2002**, *102*, 727.
66. E. E. Ross, L. J. Rozanski, T. Spratt, S. Liu, D. F. O'Brien, and S. S. Saavedra, *Langmuir*, **2003**, *19*, 1752.
67. E. E. Ross, T. Spratt, S. Liu, L. J. Rozanski, D. F. O'Brien, and S. S. Saavedra, *Langmuir*, **2003**, *19*, 1766.
68. E. E. Ross, E. Mansfield, Y. Huang, and C. A. Aspinwall, *J. Am. Chem. Soc.*, **2005**, *127*, 16756.
69. B. A. Heitz, I. W. Jones, H. K. J. Hall, C. A. Aspinwall, and S. S. Saavedra, *J. Am. Chem. Soc.*, **2009**, *132*, 7086.
70. S. May, M. Andreasson-Ochsner, Z. Fu, Y. X. Low, D. Tan, H.-P. M. d. Hoog, S. Ritz, M. Nallani, and E.-K. Sinner, *Angew. Chem. Int. Ed.*, **2013**, *52*, 749.