Reconstituting cell membrane functions with a model membrane and nanometric space

([1]*Biosignal Research Center, Kobe University*, [2]*Graduate School of Agrobioscience, Kobe University*) ○Kenichi Morigaki,[1,2]

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Biological membranes composed of lipid bilayer and associated proteins work as a platform for diverse cellular functions including signal transduction and energy conversion. We developed a model biological membrane on the solid substrate by combining a patterned lipid bilayer with a nanometric gap structure. A patterned polymeric bilayer was lithographically generated from polymerizable diacetylene phospholipid by UV irradiation.[1] Natural lipid bilayers having lateral fluidity were incorporated into the polymer-free regions by spontaneous spreading of vesicles (vesicle fusion). The polymeric lipid bilayer acted as a stable framework and the embedded fluid lipid bilayers mimicked the biological membrane with lateral mobility, two-dimensional organization, and membrane functions.

A nanometric gap structure (nanogap-junction) was created between the fluid bilayer and a polydimethylsiloxane (PDMS) sheet by attaching the surface of polymeric bilayer and PDMS using an adhesion layer with a defined thickness (e.g. silica nanoparticles).[2] The nanogap-junction having a thickness smaller than 100 nm acted as a selective and sensitive biosensing platform. From a mixture of proteins (cholera toxin and albumin), the target protein (cholera toxin) was selectively transported into the gap by the specific binding to a glycolipid (G_{M1}) in the fluid bilayer and lateral diffusion. This platform enabled to detect target molecules (e.g. biomarkers) with an elevated signal-to-noise-ratio due to the reduced background noise. Furthermore, single molecules of membrane proteins could be detected by using an adhesion layer with biocompatible polymer materials.

Patterned model membrane in combination with a nanometric gap structure can be applied to a wide variety of membrane systems and proteins. We recently succeeded to reconstitute the light harvesting machinery of the plant thylakoid membrane.[3] Furthermore, dopamine D2 receptor (D2R), a G-protein coupled receptor (GPCR) that plays critical roles in the neural functions and represents the target for a wide variety of drugs, could be reconstituted in the nanometric cleft between substrate and PDMS. These finding points to a new possibility to use a nanometric space as a platform for reconstituting and studying membrane proteins under the quasi-physiological conditions, which is difficult to be created by other methods.

1) K. Morigaki, T. Baumgart, A. Offenhäusser, W. Knoll, *Angew. Chem., Int. Ed.* **2001**, *40*, 172. 2) a) K. Ando, M. Tanabe, K. Morigaki, *Langmuir* **2016**, *32*, 7958. b) M. Tanabe, K. Ando, R. Komatsu, K. Morigaki, *Small* **2018**, *14*, 1802804. 3) T. Yoneda, Y. Tanimoto, D. Takagi, K. Morigaki, *Langmuir* **2020**, *36*, 5863.