Fabrication of Silicon-Based Planar Ion-Channel Biosensors and Integration of Functional Cell Membrane Model Systems on Solid Substrates

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Sensing

URISU, Tsuneo	Professor
TERO, Ryugo	Assistant Professor
MAO, Yangli	IMS Fellow
KIM, Yong-Hoon	Research Fellow
CHIANG, Tsung-Yi	Research Fellow
ZHANG, Zhen-Long	Graduate Student
UNO, Hidetaka	Graduate Student
SAYED, Abu	Graduate Student
NAKAI, Naohito	Graduate Student
ASANO, Toshifumi	Graduate Student
SHANG, Zhi-Guo	Graduate Student
FUJIWARA, Kuniyo	Graduate Student
SHIMIZU, Atsuko	Secretary

We are interested in the investigation of cell membrane surface reactions and the pathogen mechanism of the neurodegenerative diseases, based on the molecular science. We are advancing two subjects, aiming the creation and development of new molecular science field, "medical molecular science." One is the development of ion channel biosensor and its application to the neural network analyzer device. The other is the fundamental understanding of bilayer membrane properties using the artificial lipid bilayers on solid substrates, which is called supported bilayers, by means of atomic force microscope and fluorescence microscope-based techniques.

1. The Morphology of $GM1_x/SM_{0.6-x}/Chol_{0.4}$ Planar Bilayers Supported on SiO_2 Surfaces[1)]

Ganglioside GM1 (GM1), sphingomyelin (SM) and cholesterol (Chol) are dominant lipid components of rafts in plasma membranes. The morphology of supported planar bilayers composed of GM1, SM and Chol (Figure 1) on SiO_2 surfaces has been studied by atomic force microscopy and fluorescence microscopy. The component ratio of the SPB ($GM1_x/SM_{0.6-x}/Chol_{0.4}$) was varied in the range of x = 0–0.25.

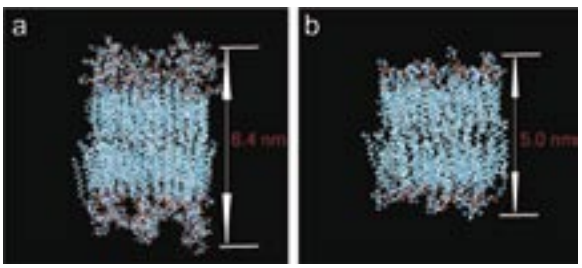

Figure 1. Membrane structure of (a) GM1/SM/Chol (1:2:2 molar ratio) and (b) SM/Chol (1:1 molar ratio) obtained by MD simulation.

The unique changes in morphology depending on the GM1 concentrations (Figure 2) are qualitatively explained by hydrogen bonding and the hydrophobic interactions between SM and Chol, and by hydrogen bonding and the steric effects between bulky GM1 headgroups under Ca^{2+} existing conditions and the electrostatic repulsion between the negative charges of GM1 headgroups under Ca^{2+} nonexisting conditions.

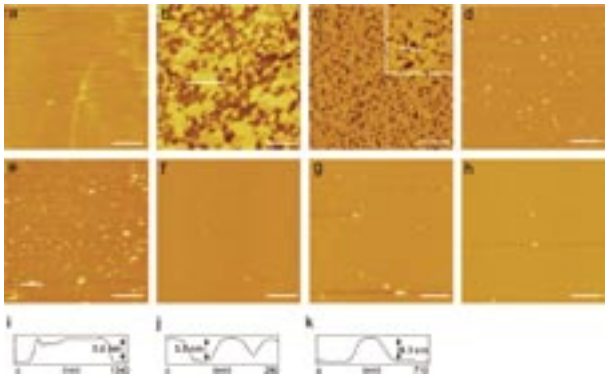

Figure 2. AFM images and line profiles of $GM1_x/SM_{0.6-x}/Chol_{0.4}$ SPBs formed on SiO_2 surface in a buffer solution containing Ca^{2+} with different GM1 content (molar ratio): (a) x = 0, (b) x = 0.05, (c) x = 0.1 (insert is magnified image (1.0×1.0 μm^2)), (d) x = 0.15, (e) x = 0.2, and (f) x = 0.25. Also, AFM images of $GM1_x/SM_{0.6-x}/Chol_{0.4}$ SPBs formed without Ca^{2+}: (g) x = 0.1 and (h) x = 0.2. (i), (j), and (k) correspond to line profiles along white lines of (b), (c), and (e). Scale bar is 1.0 μm.

2. Lipid Bilayer Membrane with Atomic Step Structure: Supported Bilayer on Step-and-Terrace TiO_2(100) Surface[2)]

Formation of a supported planar lipid bilayer (SPLB) and

its morphology on step-and-terrace rutile-TiO_2(100) surfaces were investigated by fluorescence microscopy and atomic force microscopy. The TiO_2(100) surfaces consisting of atomic steps and flat terraces were formed on a rutile-TiO_2 single crystal wafer by a wet-treatment and annealing under oxygen flow. Intact vesicular layer formed on the TiO_2(100) surface when the surface was incubated in a sonicated vesicle suspension in the condition that a full-coverage SPLB forms on SiO_2, as reported in previous studies. However a full-coverage, continuous and fluid SPLB was obtained on the step-and-terrace TiO_2(100) depending on the lipid concentration, incubation time and vesicle size. The SPLB on the TiO_2(100) also has the step-and-terrace morphology following the substrate structure precisely even though the SPLB is in the fluid phase and ~1 nm thick water layer exists between the SPLB and the substrate. This membrane distortion on the atomic scale affects the phase separation structure of a binary bilayer in micrometer order (Figure 3). The interaction energy calculated including DLVO and non-DLVO factors shows that a lipid membrane on the TiO_2(100) gains 20 times larger energy than on SiO_2. This specifically strong attraction on TiO_2 makes the fluid SPLB precisely follow the substrate structure of angstrom order.

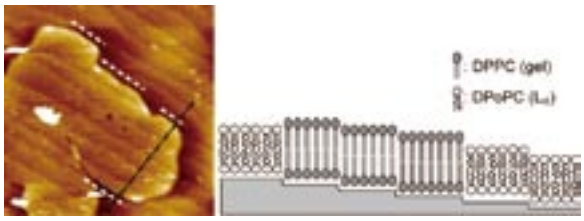

Figure 3. AFM topograph (2.0×2.0 μm^2) of the DPPC+DPoPC binary SPLB. The gel-phase domain edges running along the substrate steps are indicated by white dashed lines. The schematic illustration shows the cross-sectional view perpendicular to the substrate steps, like the black dashed-dotted line in the AFM image.

3. New Infrared Reflection Absorption Spectroscopy System for in-situ Observation of Adsorbed Biomaterials on Solid Surfaces under Water

In situ under water infrared reflection absorption spectroscopy (IRRAS) is an attractive vibration spectroscopy for biomaterials adsorbed on the IR non-transparent materials such as gold substrates, which are important in many biosensors and biochips such as DNA chips and surface plasmon resonance (SPR). We have constructed a new IRRAS system in which the sample solution is confined in the narrow space between CaF_2 prism surface and the substrate surface, and the IR beam is directed onto the substrate surface passing through the prism. The sample holders are designed to keep the thickness highly constant between the prism surface and the substrate surface during the measurements. It is essentially important, in the present system, to make sure of the condition that sample proteins are adsorbed on the substrate surface but not on the prism surface. The quite different condition in this point between fibronectin (FN) and immunoglobulin G (IgG) was well explained by the charge of the amino acid residue and the salt effects. Clear amide I bands were successfully observed under water for FN adsorbed on the SiO_2 surface (Figure 4a) and IgG on the gold surface (Figure 4b).

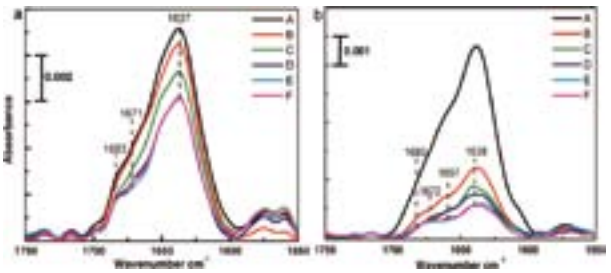

Figure 4. Amide I bands of adsorbed proteins on the substrate surfaces observed by in-situ IRRAS under D_2O solution. a) Spectra of FN using pure D_2O as the solvent; A, B, C, D, E and F. b) Spectra of IgG using NaCl (140 mM) added D_2O based PBS solution; A, B, C, D, E and F. A) Spectra for protein injection and waiting 3 h (FN) and 1h (IgG) for adsorption on solid surface. Spectra were taken every after 0.5 ml D_2O solution flushes; B) 0.50 ml C) 1.0 ml D) 1.5ml E) 2.0 ml F) 2.5 ml.

4. Development of Cell Culture Type Planar Ion Channel Biosensor for Nerve Cell Signaling Analysis[3)]

We have developed a new planar ion channel biosensor with cell culture function using SOI (Silicon On Insulator) substrate. This sensor enables not only the miniaturization of the sensor size and the high throughput screening application, but also is capable of the long time and the time lapse measurements that are difficult in the conventional any planar and pipette patch-clamp methods. The surface of SOI substrate was coated by patterned extracellular matrix (ECM), such as fibronectin and poly-L-lysine, to promote the cell adhesion and control the growth area at the preliminary designed position. TRPV1 (Transient receptor potential vanilloid type 1) channel-expressing single HEK293 cell was positioned on the micropore of the SOI substrate. Whole-cell arrangement was formed by the perforated patch using nystatin. The ion channel current was successfully measured by capsaicin stimulations. Experiments using mouse cerebral cortex cell is now under way.

References

1) Y. Mao, R. Tero, Y. Imai, T. Hoshino and T. Urisu, *Chem. Phys. Lett.* **460**, 289–294 (2008).
2) R. Tero, T. Ujihara and T. Urisu, *Langmuir* **24**, 11567–11576 (2008).
3) Z. L. Zhang, T. Asano, H. Uno, R. Tero, M. Suzui, S. Nakao, T. Kaito, K. Shibasaki, M. Tominaga, Y. Utsumi, Y. L. Gao and T. Urisu, *Thin Solid Films* **516**, 2813–2815 (2008).