

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
TERO, Ryugo
WANG, Zhihong
RAHMAN, Mashiur
ASANO, Toshifumi
NAGAIRO, Takeshi
NAGAOKA, Yasutaka
UNO, Hidetaka
NAKAI, Naohito
SHANG, Zhi-Guo
FUJIWARA, Kuniyo
SHIMIZU, Atsuko

Professor
Assistant Professor
Post-Doctoral Fellow
Post-Doctoral Fellow
Post-Doctoral Fellow
Post-Doctoral Fellow
Research Fellow
Graduate Student
Graduate Student
Graduate Student
Graduate Student
Secretary

We are interested in the investigation of cell membrane surface reactions and the pathogen mechanism of the neuro-degenerative 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. Development of Neural Network Device and Precise Microfabrications

We have successfully developed the neural cell device which emits the action potential by the photo-stimulation using a photo-receptor ion channel protein and the ion channel biosensor as the detector of the action potential. As an application of these elementary devices, we are now developing the neural cell network devices, using the state-of-the-art precision work combining the superprecision machining, the hot emboss technology, and the LIGA process. The high precision fabri-

cation technology will bring a significant breakthrough into the brain science.

2. Surface-Induced Phase Separation of Sphingomyelin/Cholesterol/Ganglioside GM1-Planar Bilayer on Mica Surfaces and Microdomain Molecular Conformation that Accelerates A β Oligomerization

Ganglioside GM1 mediates the amyloid beta (A β) aggregation that is the hallmark of Alzheimer’s disease (AD). To investigate how ganglioside-containing lipid bilayers interact with A β , we examined the interaction between A β 40 and supported planar lipid bilayers (SPBs) on mica and SiO₂ substrates using atomic force microscopy, fluorescence microscopy, and molecular dynamics computer simulations. These SPBs contained several compositions of sphingomyelin, cholesterol, and GM1 which covers compositions commonly seen in eukaryotic biomembranes and were treated at physiological salt concentrations. Surprisingly high speed A β aggregations of fibril formation were induced for all GM1 concentrations examined on the mica surface, but only globular agglomerates are formed slowly on the SiO₂ surfaces. Especially for the 20 mol% GM1 concentration on the mica surface, unique triangular domains were formed and the high speed A β aggregations were observed only outside of the triangular domains. We have found that some unique surface-induced phase separations are induced due to the GM1 clustering effects and the strong interactions between the GM1 head group and the water layer adsorbed in the ditrigonal cavities on the mica surface. The speed of A β 40 aggregation and the shape of the agglomerates depend on the molecular conformation of GM1, which varies depending on the substrate materials. We identified the conformation that significantly accelerates A β 40 aggregation, and we think that the detailed knowledge about the GM1 molecular

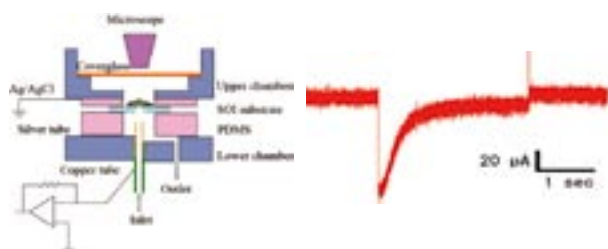


Figure 1. Structure of ion channel biosensor, and the observed channel current gated by laser irradiation. ChR2 is expressed on the C2C12 cell set on the biosensor substrate.

conformation obtained in this work will be useful to those investigating A β -GM1 interactions.¹⁾

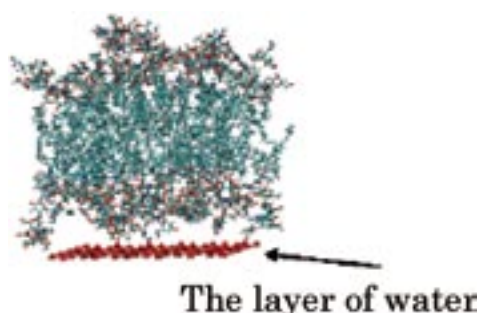


Figure 2. Starting model of the calculation, including the substrate effects. CHOL/GM1 (50:50)-SPB sitting on a layer of water molecules trapped in the ditrignonal cavities with 0.52-nm spacing of the mica surface.

3. Clustering Effects of GM1 and Formation Mechanisms of Interdigitated Liquid Disordered Domains in GM1/SM/CHOL-Supported Planar Bilayers on Mica Surface

We investigate the formation mechanisms of the interdigitated liquid disordered domain (ILDD), which is observed in the ganglioside (GM1)/sphingomyelin (SM)/cholesterol (CHOL) bilayers on a mica surface and accelerates the formation of fibrillar A β agglomerates, using molecular dynamics computer simulations and atomic force microscopy. The ILDD structure is stable both on mica and SiO₂ surfaces, but it is observed only on the mica surface. We conclude that the phase separation of SM- and GM1-rich domains is induced by GM1 clustering and the interaction between the GM1 head group and the water layer adsorbed in the ditrignonal cavity on the mica surface.

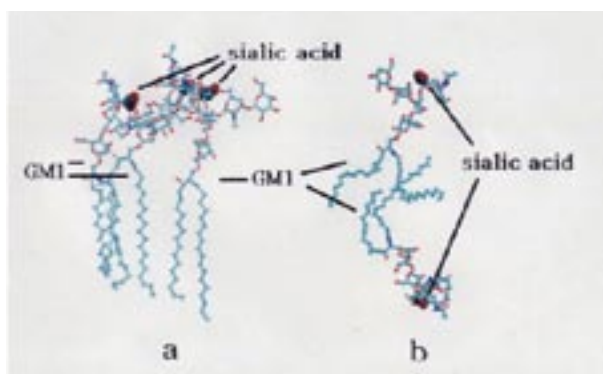


Figure 3. Molecular structures of GM1 and SM in the lipid bilayer of GM1_x/SM_{32-x}/CHOL₃₂ on the mica surface, for $x = 16$ (a) and 24 (b) after 10-ns simulation.

4. Anomalous Diffusion in Supported Lipid Bilayers Induced by Nanostructures on Substrate Surfaces

Lateral organization and diffusion of lipids and membrane proteins are crucial factors of biological reactions on cell membranes such as signal transduction and cell recognition. We have observed directly the lipid diffusion in supported lipid bilayers (SLBs) by single molecule tracking (SMT) method. SMT measurement of fluorescent dye-labeled lipid (lissamine rhodamine B labeled dipalmitoyl- phosphatidylethanolamine; Rb-DPPE, $E_x/E_m = 557 \text{ nm}/571 \text{ nm}$) in dioleoylphosphatidylcholine (DOPC)-SLBs on thermally oxidized SiO₂/Si(100) and step-and-terrace TiO₂(100) surfaces was performed by diagonal illumination method applying an objective type total internal reflection fluorescence microscope. The SiO₂ surface has amorphous structure of $\sim 1 \text{ nm}$ at peak-to-valley. The step-and-terrace TiO₂(100) surface has linear atomic steps at 550 nm interval, and $50\text{--}300 \text{ nm}$ oval pits surrounded by an atomic step exists in terraces. Diffusion of Rb-DPPE in the DOPC-SLBs on SiO₂/Si(100) and TiO₂(100) surfaces were recorded at the time resolution ranging from $497 \mu\text{s}$ (2011 fps) to 30 ms (33 fps), and the diffusion coefficients on various time scale were evaluated from the time evolution of mean square displacement ($\langle r^2 \rangle$). The diffusion coefficients (D) of Rb-DPPE decreased with diffusion length, and their tendency depend on the substrate nanostructures (Figure 4). On TiO₂ (100) decrease in diffusion coefficient (D) is observed at the mean diffusion distance ($\sqrt{\langle r^2 \rangle}$) less than 400 nm , which corresponds to the interval of the atomic steps. We attribute this anomalous diffusion to the surface atomic steps on the TiO₂(100) surface.

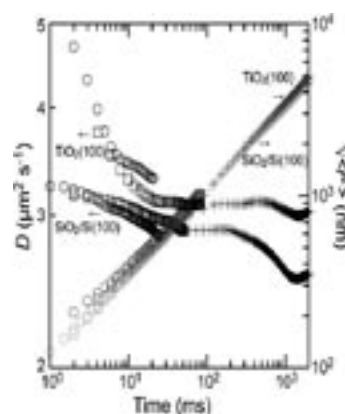


Figure 4. Time dependence of the diffusion coefficients (D) of Rb-DPPE and the mean diffusion distance ($\sqrt{\langle r^2 \rangle}$).

Reference

- 1) Y.-Li. Mao, Z.-G. Shang, Y. Imai, T. Hoshino, R. Tero, M. Tanaka, N. Yamamoto, K. Yanagisawa and T. Urisu, *Biochim. Biophys. Acta, Biomemb.* **1798**, 1090–1099 (2010).

Awards

TERO, Ryugo; CSJ Presentation Award 2010.

UNO, Hidetaka; 3rd International Symposium of Nano-medicine, Young Scientist Award.