lipid molecules and/or hydrogen-bond making and breaking dynamics with water molecules. Therefore, terahertz time-domain spectroscopy (THz-TDS) is a powerful tool to investigate lipid molecule dynamics. In this study, we focus on dependence of the low-frequency dynamics of lipid bilayers on hydration, temperature, and their structure by using THz-TDS. As a sample, we selected 1, 2-Dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG), which is known to form several different structures like crystalline or gel phase states. We measured the temperature dependence of the low-frequency spectra of the DMPG under different hydration degrees and structures. The absorption coefficients of each sample were obtained in the frequency region from 10 cm⁻¹ to 50 cm⁻¹. We discuss about the dynamics of DMPG from the changes of the absorption coefficients.

3PT157 表面張力レプリカ交換法による脂質二重膜の構造サンプリング

Surface tension replica exchange molecular dynamics method for an efficient sampling of lipid bilayer structures

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Generalized ensemble algorithm is one of the efficient sampling methods for biomolecular systems. Recently, various replica exchange molecular dynamics methods (REMD) in different parameter spaces have been developed. However, simulations for membranes or protein-membrane systems are still difficult with generalized ensemble algorithms, because the membrane can easily collapse in such enhanced sampling methods like temperature REMD. To overcome this problem, we propose a new replica exchange molecular dynamics method, where the surface tension of the system in an NPyT ensemble is exchanged (γ REMD). Exchanging the surface tension can moderately induce the deformation of lipid bilayers. We introduced γ REMD in our laboratory-developed MD program (GENESIS), and it was applied to a fully hydrated 1,2-di-palmitoyl-phosphatidylcholine (DPPC) lipid bilayers. We found that the area per lipid and membrane thickness can change significantly in the γ REMD simulation, compared to the single NPyT simulation. We believe that our method is useful for investigating structural properties of lipid bilayers, conformational changes of membrane proteins in membranes, and protein-lipid interactions.

3PT158 膜厚変化とゲル、アミノ酸、水素化アモルファスシリコンを用いたイオン伝導整流素子の特性

Film thickness and property of ion conductive rectification element using amino acids, gel and hydrogenated amorphous silicon

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This report mainly focuses an electro-potential-current property correlated with film thickness on ion conductive rectification element using amino acids, gel and hydrogenated amorphous silicon. In general, ionic conduction, in solution as well as in intermediate states between a liquid and a solid, such as gels, is a phenomenon key to inter-conversion between light energy, chemical energy, and electric energy. As such, this phenomenon is essential for devices that store, generate, and convert electric energy, including batteries, photovoltaics, capacitors, and actuators. This report secondly focuses on ionic conduction in nontoxic materials to discuss the creation of elements coated with multi-layer functional thin-films using a combination of different technologies, such as the rectification property of ionic conduction, photo-induction control, and the use of wavelength conversion materials, and the potential application of these materials to biological tissue. Ion conductive behaviors of neutral, acidic, or basic amino acid dispersed in agarose gel are characterized. We report an attempt of measuring a photo-controlled effect of a-Si:H film on ion conducting behavior of bio-molecules in detail, comparing several thickness of a-Si:H film.

3PT159 BLM を介したイオン性蛍光プローブの膜透過性—電流と蛍光に基づいた動的解析—

Permeability of an ionic fluorescent probe through a BLM - dynamic analysis based on an electric current and a fluorescent change-

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Transport of an ionic species through a bilayer lipid membrane, BLM, is influenced not only by its hydrophobicity but also by a coexisted counter ion, and the transport mechanism has been considered to be complicated. Recently, new opinion for the BLM transport of an ion has been reported by Shirai et al. [1, 2]. In the opinion, a BLM was recognized as like a thin liquid membrane with two aqueous/membrane interfaces. The transferring ion distributes to a BLM with coexisted counter ion and transports across the BLM keeping electroneutrality in the BLM. Electrochemical results were given as experimental evidences for the transport mechanism, but not enough for supporting the cotransport mechanism which is not detected by electrochemical measurement. In the present work, an ionic fluorescent probe was employed as the transferring ion through a BLM. Transport of the probe was detected not only as the ion transfer current but also as fluorescent change near to the BLM induced by the ion transport. Comparing the transport current with the fluorescent change, whether the transferring species is ionic or neutral was estimated. According to the observation, the mechanism of ion transport through a BLM was discussed. [1] O. Shirai, S. Kihara, Y. Yoshida, M. Matsui: J. Electroanal. Chem., 389, 61-70 (1995) [2] O. Shirai, Y. Yoshida, M. Matsui, K. Maeda, S. Kihara: Bull. Chem. Soc. Jpn., 69, 3151-3162 (1996)

3PT160 ABC トランスポーターのヌクレオチド結合ドメインに対する ATP 結合自由エネルギー計算

Calculation of the binding free energy of ATP to the nucleotide binding domain of an ABC transporter

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ABC (ATP-binding Cassette) transporters exist in almost all kinds of living things and use ATP as an energy source to transport a wide range of substrates across the membrane. ABC transporters have the common 3D structural architecture: the whole molecule is divided into two domains, that is, Transmembrane Domain (TMD) and Nucleotide-Binding Domain (NBD). NBD has two highly conserved ATP binding sites (Walker A&B and LAGGQ). It has been proposed that binding of ATPs to these sites induces the dimerization of two NBDs, eventually leading to a large structural change from the inward- to outward-facing conformation of TMD.

Our study is to elucidate this structural change mechanism from energetic aspects. In this study, as a first step, we evaluated the free energy gain obtained from binding of the ATP to NBD. We examined this for NBDs in the maltose transporter (MalFGK₂) from *Escherichia coli*. For this purpose, molecular dynamics (MD) simulations were performed starting from the crystal structure of the NBD dimer and subsequently the above free energy was calculated according to the MM/PBSA method.

We first evaluated the binding energy of one ATP molecule to an NBD monomer. The resultant values were -37.6 and 4.1 kcal/mol when ATP binds to the Walker A&B motif or the LSGGQ one, respectively. Next, the NBD dimerization energy was evaluated to be -5.91 kcal/mol. We are now calculating the binding free energies of ADP and inorganic phosphate (Pi) to an NBD to elucidate the role of ATP hydrolysis in the substrate transportation process.

3PT161 パターン化モデル生体膜へのロドプシンの再構成

Reconstitution of rhodopsin into micropatterned model biological membrane

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Rhodopsin (Rh) is a photoreceptor that forms a complex with transducin (G_t) upon activation by light. It has been reported that the affinity of Rh*-G_t complex to lipid rafts (raftophilicity) was higher than that of Rh (Hayashi et al. ARVO meeting (2011)). We are developing a methodology to evaluate the raftophilicity quantitatively with a supported phospholipid bilayer (SPB). Micropatterned SPBs composed of photo-polymerized lipid bilayer and fluid lipid bilayer. By changing the compositions of polymeric and fluid bilayers, liquid ordered (l_o) domains and liquid disordered (l_d) domains in the fluid bilayer were separated into a pre-determined pattern. To measure the localization of Rh and Rh*-G_t in l_o regions, we incorporated Rh into SPB by rapid dilution of Rh/ octylglucoside micelles. We confirmed single Rh molecules in SPB by TIRF-microscopy. Furthermore, we observed binding of fluorescently labeled α -subunit of G_t (G_{at}) upon activation of Rh and changes in

the mobility of Rh*-G_i upon subsequent addition of GTP. Hence, we concluded that Rh and G_i in SPB were active. At the same time, we also observed some immobile Rh molecules in SPB, which could interfere with the analysis of raftophilicity. We discuss on the reconstitution strategies of Rh to establish the means to measure the raftophilicity in micropatterned *I_o* domains.

3PT162 カリウムチャンネル KcsA の開閉によるリン脂質の flip-flop 誘起能の評価

Induction of phospholipid flip-flop by KcsA channel gating

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Regulation of phospholipid flip-flop is important for homeostasis of biological membranes. Flip-flop of phospholipids in the endoplasmic reticulum (ER) is very fast, compared to that in other membranes. However the flippases that govern the fast flip-flop in the ER have not been identified, and the mechanism by which the flip-flop is induced is still unknown. It has been reported that KcsA induces flip-flop of phospholipids. KcsA is a pH-dependent potassium channel, activated by acidic pH. Conformational change of transmembrane region during channel gating was recently observed at a single channel level. We therefore hypothesized that the fluctuation of transmembrane region influences flip-flop of phospholipids.

Dithionite reduction assay was used to measure flip of C₆-NBD-phosphatidylcholine (C₆-NBD-PC) in proteoliposomes, composed of PC and KcsA. KcsA was found to induce flip of phospholipids at acidic pH, while less affected at neutral pH. To confirm whether the fluctuation of transmembrane region of KcsA induce flip, we measured flip of C₆-NBD-PC at acidic pH in the presence of tetrabutylammonium, which locks KcsA in the open state. The results showed that KcsA mediates flip of phospholipids even in the open-locked state. These results suggest that KcsA enhances flip of phospholipids not by the fluctuation of transmembrane region, but by the open structure or the change of charged state of amino acids in transmembrane region.

3PT163 人工細胞膜アレイを用いた全自動イオンチャンネル計測システム

Automated Drug Screening System for Ion Channel Proteins using Artificial Cell Membranes

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This paper describes an automated drug screening system for Ca²⁺-activated K⁺ channel (BK channel). BK channels are pharmacological targets for the treatment of stroke. We have been developing multiple lipid bilayer membranes (BLMs) system for reconstituting the membrane proteins. In this study, we are succeeded in simultaneous reconstitution of BK channels in the BLMs array using an injection robot, and the channel inhibition behavior was examined using K⁺ channel blocker. Our system can be applied to a high-throughput screening (HTS) system for drug discovery. Membrane proteins account for approximately 25% of the polypeptides encoded within the human genome. Of these, ion channels are an important class of proteins that mediate ion flow across cellular membranes and which, among other things, are responsible for the excitability of neurons. In the drug discovery, the high-throughput screenings of the ion channels are highly desirable. We have been developing the lipid bilayer membrane array for the HTS of the ion channels using MEMS technology. BK channels are essential for the regulation of several key physiological processes including smooth muscle tone and neuronal excitability. We have therefore attempted to reconstitute BK channel within the artificial lipid bilayers. In this study, BK channels were automatically reconstituted in the BLMs array device by using an injection robot, and also the channel activity was examined using its inhibitor.

3PT164 KcsA カリウムチャンネル活性に影響を及ぼすリン脂質作用部位の同定 Identification of the interaction site with the effective phospholipids for the KcsA potassium channel activity

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Membrane lipids are known to affect the function and activity of ion channels. Specific lipid molecules are indispensable for some ion channels to become active. How the specific lipids regulate the ion channel activity is essential to

understand the structure-function relationship of ion channels in the membrane environment. For the KcsA potassium channel, anionic phospholipids such as phosphatidylglycerol (PG) have been thought to play an essential role in its activity as the KcsA channel exhibits quite low open probability in the absence of these lipids. Our previous study revealed by means of single-channel current recordings in the asymmetric lipid bilayer that the KcsA channel requires PG molecules in the inner leaflet to exhibit high open probability. In this study we tried to identify the interaction site with PG within the KcsA molecule for further understanding of lipid-mediated regulation of the KcsA channel activity. Potential positive charges such as Arg, Lys and His were expected to be candidates for the interaction site with PG. We found that mutation of several sites out of 24 potential positive charges in the monomeric KcsA molecule were sensitive to the PG effect on the KcsA channel activity. Mechanism underlie PG-mediated regulation of the KcsA channel opening will be discussed.

3PT165 Kv1.2 チャンネルでの選択的イオン透過

Selective Ion Permeation through the Kv1.2 Channel

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Ion selectivity is a crucial function for ion channels. So far, it has been considered the K⁺ channels discriminate for K⁺ over Na⁺ more than a thousand-fold due to the destabilized binding of Na⁺ at the selectivity filter. However, several recent researches showed that Na⁺ can enter into the selectivity filter and permeate: the x-ray crystallographic structure of the K⁺ channel with Na⁺ was resolved, where Na⁺ ions form six-coordination structure in the selectivity filter. Its structure was then supported by theoretical calculations. The electrophysiological measurement also suggested that a certain amount of Na⁺ ions can permeate through the K⁺ channel by punch-through mechanism. Thus, there still exists controversial arguments concerning the ion selectivity. Here we observed the efflux of ion in the mixed ion solution, 200 mM KCl and 200 mM NaCl, through the Kv1.2 channel by the molecular dynamics simulation. It is found that K⁺ ions permeate at rates at about five times higher than Na⁺ ions, which is approximately the same ratio the punch-through mechanism reported. We also found that once ions, regardless of ion species, enter into the central cavity, they cannot return to the cytoplasmic side solution. This means the selectivity is caused by the free energy barrier at the entrance of the channel. Therefore, the selectivity is a part of ion permeation issues rather than the stability of ions in the channel; kinetics, instead of thermodynamics, determines the ion selectivity.

3PT166 Controlling an ion channel's voltage sensing domain without voltage

Morten Bertz, Kazuhiko Kinoshita (Waseda University, Dpt. of Physics)

Voltage gating - the opening and closing of ion channels on response to changes in membrane potential - is fundamental to signal transduction in living organisms. Voltage gating is achieved by four voltage sensing domains (VSDs) that surround the ion-conducting pore of the channel protein. In these VSDs, conserved positively charged residues located in the S4 transmembrane helix move according to the transmembrane electrical field, and the resulting conformational change is transmitted to the channel pore. Up to date, however, direct structural information on the deactivated, closed state of a channel has remained elusive. Consequently, the extent and direction of the VSD movement remain controversial, with both small translations and large-scale movements reported in the literature depending on the technique used in the investigation. Here, we attempt the manipulation of voltage sensor conformation using engineered probes as a substitute for voltage to shed light on the transitions involved in voltage gating.

3PT167 ガラス針による人工平面膜へのイオンチャンネルの再構成

Reconstitution of ion channel into lipid bilayer using glass needle

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Ion channels are membrane proteins that regulate cell functions by controlling ion flow through cell membrane. To elucidate the molecule mechanism of the regulation for the ion flow, it is necessary to investigate the relationship between the conformation and function and interaction with some factors. To aim this task, ion channel is kept suppressing lateral diffusion in the membrane for