2P161**Reconstitution of bacteriorhodopsin into artificial cyclic phospholipid membrane**○Masashi Sonoyama¹, Shigeki Mitaku¹, Motonari Shibakami², Shin Miyoshi²¹Dept. Appl. Phys., Nagoya Univ., ²AIST

Cyclic lipids consisted of tetraethers of glycerols are characteristic of Archaea that live in extreme environmental conditions. The cyclic structure with the ether bonds is thought to be essential for the remarkable resistance of Archaea to extreme situations. Shibakami and coworkers designed and synthesized an artificial cyclic phospholipid with tetraethers of glycerols and diacetylenic alkyl chains, and studied its self-assembly to microtubules (Miyawaki, Goto and Shibakami, Chem. Lett. 32, 1170-1171 (2003)). In this study, reconstitution of bacteriorhodopsin into cyclic phospholipid vesicles was carried out for investigating effects of structural properties of cyclic phospholipids on structure, function and dynamics of membrane proteins. Bacteriorhodopsin was successfully incorporated into cyclic phospholipid liposome by mixing solubilized sample with a mild nonionic detergent, Triton X-100 and cyclic lipid membrane, and subsequent detergent removal by Bio-beads. The proteoliposome of bacteriorhodopsin and cyclic phospholipid showed significant red shift of visible absorption upon irradiation with visible light, suggesting light-adaptation of bacteriorhodopsin in cyclic lipid vesicles. Experimental results on structural properties and function of the proteoliposome will be discussed.

2P163**Effect of Ion Concentration on Regeneration of Bacteriorhodopsin from Bacterio-opsin and All- *trans* Retinal**

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Bacteriorhodopsin (bR) has seven transmembrane helices similar to a G-protein coupled receptors (GPCRs), which specifically binds the ligand. Bacterio-opsin (bO) can be readily regenerated to the active form of bR by the spontaneous binding of all-trans retinal. This regeneration of bR may be a good model for the ligand binding of GPCRs. Recently we carried out the regeneration experiments of bacteriorhodopsin at high temperature in which two regeneration components were observable, suggesting that bO in the thermally unfolded state regenerates to bR by dynamic structural fluctuation [1],[2]. In this study, we carried out the regeneration experiments, changing pH and ionic concentration for understanding the contribution of electrostatic interactions on regeneration of bR. The results showed that in alkaline and acid condition, structural fluctuation of bO which is monitored by the degree of regeneration was observed from lower temperature (35 °C) than pH 7. When NaCl was added to the solution, the characteristic time of the regeneration tended to slow down. Based on the experimental results, we will discuss the characteristics of the structural fluctuation of bR in the high temperature intermediate state. References[1] G. D. Ghimire, H. Sugiyama, M. Sonoyama and S. Mitaku, Biosci. Biotechnol. Biochem. 2005, 69, 252-254. [2] H. Sugiyama, M. Sonoyama, G. D. Ghimire and S. Mitaku, Biosci. Biotechnol. Biochem. 2006, 70, 1350-1355.

2P162**Kinetic Study for Irreversible Photobleaching of Bacteriorhodopsin in Purple Membrane at Alkaline pH**

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Bacteriorhodopsin (bR), found in purple membrane (PM) of *Halobacterium salinarum*, is a light-driven proton pump. The function of bR is achieved along a sequential structural change to several photo-intermediate states, and the ground state of bR can be spontaneously recovered after that. Our denaturation experiment of bR in PM showed that bR undergoes an irreversible bleaching above 60 °C under illumination, while it is stable below 70 °C in darkness, suggesting that a restoring force during the photocycle became weakened at this temperature range. Kinetic study for the photobleaching of bR in PM at high temperature exhibited an existence of two kinds of bR species upon light illumination. Furthermore, the biphasic kinetics altered to the monophasic one above pH 10, accompanied with the structural changes with elevating pH. However, it is still unknown what is a cause of the fast and slow components. Here, we elucidated a temperature dependency of decay constants of each component during the photobleaching by varying pH. Arrhenius plot showed that the slopes of two lines correspond to the fast components of the photobleaching at pH 7 and 9 were similar, while the slope due to the slower one at pH 9 was larger than that at pH 7, suggesting a different path of the photobleaching even in the same biphasic decay. Further, the slope due to the photobleaching at pH 11 was similar to that of the slow component at pH 9 rather than the faster one. A model for the fast and slow bleaching process will be discussed from these results.

2P164**Live cell imaging of GLUT4 molecules in adipocytes using fluorescent quantum dot and TIRF microscopy**

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To acquire information on insulin dependent changes in GLUT4 movement, we have developed a method of labeling the GLUT4 molecule with a fluorescent quantum dot, in which myc-tagged GLUT4-eCFP stably expressed in 3T3L1 adipocytes were labeled with Qdot conjugated with Fab fragment of anti-myc antibody. In 2005, we tracked the movements of GLUT4 molecules in 3T3L1 adipocytes using real-time confocal microscopy and reported movement changes, in response to insulin, using Qdot labeled GLUT4 molecules. This year, to further elucidate insulin dependent changes in GLUT4 behavior, we observed GLUT4 molecule dynamics at the plasma membrane using a TIRF microscope. TIRF microscopy revealed insulin to increase the number of GLUT4 molecules approaching the plasma membrane by 3.75 fold, which is consistent with our previously reported observation that insulin increases the population of GLUT4 molecules moving rapidly in the cytosol. Insulin also increased the time this molecule stayed at the plasma membrane by 1.4 fold, and this increase is mostly attributable to the increased fraction of GLUT4 molecules staying at the plasma membrane for more than 10sec. Together with the results reported previously, these observations indicate that insulin regulates both the step whereby GLUT4 vesicles are released from the storage compartment and the fusion step at the plasma membrane.