- 1P322 セロトニン受容体に特異的なリガンド検出用バイオセンサー
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- 1aK07 A Sensitive Biosensor for Specific Ligands of 5-Hydroxytryptamine type-3 receptor
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We have developed a subtype specific ligand sensor for 5-hydroxytryptamine (5-HT, serotonin) receptor subtype 3. There are 14 subtypes so far defined in the 5-HT receptor, and each of them shows their over tissue distribution, pharmacological and physiological functions. In drug development, we need to discriminate the subtypes to avoid unfavorable side effects. We genetically engineered polypeptides corresponding to the ligand binding domain of 5-HT₃ receptor subunit: 5-HT_{3A}bp and 5-HT_{3B}bp. We examined the effect of the proteins on 5-HT-induced inward currents in *Xenopus* oocytes co-expressed heteromeric 5-HT₃ receptors. 5-HT_{3A}bp or 5-HT_{3B}bp applied together with 5-HT inhibited the inward currents in a dose-dependent manner. This clearly indicates that 5-HT_{3A}bp and 5-HT_{3B}bp can efficiently bind 5-HT. We immobilized the polypeptide at the base of the Au electrode and detected specific ligands of 5-HT₃ receptor by surface plasmon resonance (SPR). We succeed in developing the biosensor for the ligands of 5-HT₃ receptor with the polypeptide which has ligand-specific binding ability. This work was supported financially by National Institute of Advanced Industrial Science and Technology (AIST) Upbringing of Talent in Nanobiotechnology Course, Promotion Budget for Science and Technology from Ministry of Education, Culture, Sports, Science and Technology.

- 1P324 リポソーム環境での膜タンパク質の合成と機能化
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- 1aK08 Functional Membrane Protein Expression and Integration on Lipid Vesicles.
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Cell-modeling research has been attracting increasing interests, as a potent methodology to understand the structure and function of living cells. We describe here the recent development on the studies toward the construction of an artificial-cell-model. We have been studied direct constitution of cell-free synthesized membrane proteins to giant liposomes. As a model membrane protein, apo cytochrome b5 (b5) and connexin (Cx: intercellular gap-junction channel forming protein) were adopted. The localization of the membrane proteins (GFP-tagged) on the liposome was investigated by confocal microscopy. The proteoliposome was isolated by simple centrifugation of the reaction mixture. In the case of b5, conjugated dihydrofolate reductase (DHFR) was synthesized in the same procedure and the protein was directly displayed on the liposome surface. In the case of Cx43, hydrophilic dye (calcein) transfer from the proteoliposomes to cultured cells was observed. The transfer was critically blocked by GJ inhibitor. This supports that the synthesized Cx was functional to form GJs between liposome and the cell. We demonstrate here a new example for directly obtaining proteoliposomes by cell-free protein synthesis. This method should create new opportunities for the formation and analysis of functional membrane proteins.

- 1P323 分子通信への適用を目指したチャネル形成タンパク質組込膜小胞の作成
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- Channel Forming Protein Integrated Vesicle Creation for Molecular Communication
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Molecular communication is a new communication paradigm that allows nano-to-micro size senders and receivers (e.g., cells) to communicate using molecules as an information carrier. In order to achieve transportation of molecules between senders and receivers in molecular communication, vesicles that can exchange molecules with senders (or with receivers) may act as a container of molecules between senders and receivers. This abstract describes research in creating connexin-43 (one of the intracellular gap-junction channel forming proteins) integrated vesicles. We investigated three methods to create the connexin-43 integrated vesicles. The first method is a direct constitution of cell-free synthesized connexin-43 to vesicles, the second method is a direct formation of connexin-43 integrated vesicles from cells, and the third is a reconstitution of connexin-43 extracted from cells to vesicles. We successfully created the connexin-43 integrated vesicles through the first and the second methods (the direct constitution and the direct formation methods). The localization of the connexin-43 (GFP-tagged) on the vesicles in each method was investigated by confocal microscopy. We are currently trying to create the connexin-43 integrated vesicles through the reconstitution (the third method). We are also experimentally examining the functionalities of the connexin-43 integrated vesicles created by each method through transferring hydrophilic dye from the vesicles to cells.

- 1P325 昆虫ウイルス由来タンパク質ポリヘドリンを用いた外来タンパク質固定化技術の開発
発
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- Immobilization of diverse foreign proteins in viral polyhedra
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The immobilization of biologically active molecules is important for diagnostic screening as well as for micro-biotechnology. Protein immobilization methods for high-throughput screening have been paid much attention because proteins play key roles as structural elements in the biological body. Aiming to realize the protein microarray which enables us high-throughput analysis of protein interactions, we developed a method of immobilization of protein based on an insect virus strategy.

Cypoviruses are insect viruses that produce a crystalline particle called polyhedron in which progeny virion is occluded. The polyhedra produced by some insect viruses are known to be useful platform for protein immobilization without lost of biological activity. Previous works showed that *Bombyx mori* cypovirus (BmCPV) outer capsid protein, VP3, was the immobilization signal for incorporation of protein molecules into polyhedra. Fusion of the enhanced green fluorescent protein (EGFP) to the C-terminal of VP3 resulted in its incorporation into polyhedra when the chimeric protein co-expressed with polyhedrin during BmCPV infection.

In this study, we focus on spectroscopic analysis for protein-protein interactions and biological activities. Interaction between the protein and the tagged with EGFP protein was observed by fluorescence spectroscopy. Our observations indicate that the immobilized protein molecules hold their biological activities on the surface of polyhedra. We also discuss the possible application of our method for protein microarrays.