論 文 の 内 容 の 要 旨

論文題目　Cell-Free Synthesis of the Human Membrane Protein: The Chemokine G-Protein Coupled Receptor
（ヒトケモカイン受容体の無細胞合成の研究）

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Summary

Membrane proteins are the prime targets for drugs and currently they account for 50% of all the drugs in the market. However, despite their significance in combating disease, the heterologous expression and purification of membrane proteins in sufficient amount for functional and structural analysis still remains a challenge.

The G-protein coupled receptors (GPCRs) are membrane proteins characterized by their hydrophobic seven transmembrane (7TM) and they are mainly involved in intracellular signaling. The human genome comprises of more than 800 GPCRs with distinct functions. The chemokine GPCRs mediate the movement of leukocytes to inflammation sites and a malfunction in the expression of these receptors is associated with asthma, cancer, HIV/AIDS, neural and heart disease.

Here, we investigated the expression, solubility, ligand binding and G-protein activation of the chemokine receptor, CX_3CR1, in the presence of a nanodisc using a reconstituted cell-free translation system, the PURE system. A productivity of 12-28ug/ml with more than 90% solubility was achieved which is sufficient for functional studies. Hence, we measured the binding affinity of CX_3CR1 and obtained a 3.47nM Kd value. In addition, we analyzed the G-protein activation of the receptor in the presence of G-proteins and the activity increases upon addition of a ligand. Therefore, cell-free reconstitution has a significant advantage in developing antibodies for disease caused by inappropriate expression of GPCRs.