DATE: Day 10 Month May Year 2019

SUMMARY of
2018 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Reconstitution and characterization of membrane-containing protein macromolecular assembly
Applicant	**Name**	**Naoko Mizuno**
	Affiliation	**Max Planck Institute of Biochemistry**
	Present Title	**Group Leader**
Research Collaborator (Host PI)		**Junichi Takagi**

Summary

Cell surface receptors are critical gateways to sense outside environments and to trigger cellular signaling pathways, resulting in appropriate cellular responses. These responses include cell growth, survival, migration and polarization. Failure in proper receptor activation leads to developmental defects and contributes to the formation of aggressive cancers. Hence, understanding organization and functions of cell surface receptors on a molecular level is the basis for counteracting detrimental effects. Recent technical developments in the field of cryo-EM helped to advance our understanding of critical receptors, however, several types of receptors have still been proven to be extremely challenging to obtain structural information due to their conformational flexibility.

The Mizuno lab at the Max Planck Institute of Biochemistry in Germany studies the molecular mechanisms governing the change of cell shape by bottom-up reconstitutions using purified recombinant protein components. We employ a systematic and hierarchical assembly of protein components to eventually analyzing cytoskeleton remodeling and mimicking of cell shape reorganization in a stepwise fashion. We use a combination of cutting-edge techniques of hybrid structural biology (cryo-EM and X-ray crystallography), light microscopy and biochemical/biophysical approaches. Our core expertise is cryo-EM, which has emerged as the method of choice to study the molecular mechanisms of (super-)macromolecular assemblies. The host researcher (Professor Junichi Takagi) is a world expert of receptor structural biology and biotechnology. The Takagi lab developed a series of cell receptor antibodies and designed small peptide-motifs that are aimed to fix conformations of receptor proteins facilitating the structural investigation of membrane proteins. We therefore aim to combine our strengths and address various questions in the field of receptor biology and signaling.

From Mizuno's lab, the PI and a PhD student visited Takagi's lab from July 2nd to 31st in 2018. With the help of Dr. Kyoko Matoba at Takagi's lab, the student learned necessary techniques of how to handle tissues and extract proteins, and to produce antibodies for affinity purification. The experimental procedures were established and optimized during the visit of the student in the host laboratory and then, successfully reproduced in Germany. Currently, further optimizations of experimental procedures are ongoing in order to scale up the yield of the target proteins. Furthermore, experiments towards a structural analysis were initiated. In the future, we will continue to attempt gaining structural insights and to elucidate the crosstalks of the protein-protein networks.