DATE: Day 18 Month May Year 2017

SUMMARY of

2016 RESEARCH RESULTS REPORT

For International Collaborative Research with IPR, Osaka University

Research Title		**STRUCTURAL STUDIES OF MEMBRANE VIRAL CHANNELS USING NMR**
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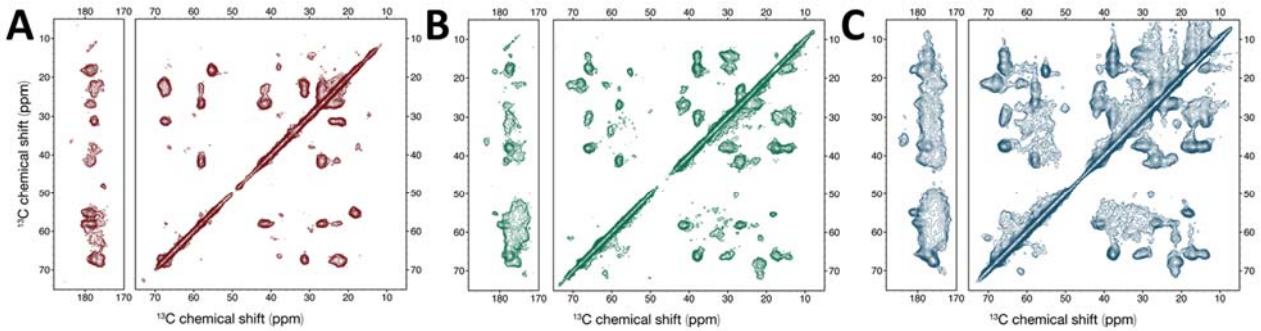
	Present Title	**Associate Professor**
Research Collaborator (Host PI)		**Toshimichi FUJIWARA (Professor)**

Summary

Viroporins are viral ion channels that interact with multiple viral and host proteins, and are currently modified to produce live attenuated vaccines (LAVs). For rational design of LAVs, a precise structural knowledge of the critical residues and/or domains of the protein is required.

The envelope (E) and the small hydrophobic (SH) proteins are critical viroporins in the severe acute respiratory syndrome coronavirus (SARS-CoV) and in the human respiratory syncytial virus RSV (hRSV), respectively. Although structures of these viroporins are available, they are still of low resolution, and have been obtained in detergents. Our objective is to characterize them in lipid membranes using solid-state NMR.

Isotopically $^{13}C^{15}N$-labeled protein (SARS-CoV E or SH protein) were reconstituted in DMPC. Both wild type SARS-CoV E (A) and SH proteins (B) were reconstituted at a ratio of 1 pentamer per ~100 lipid molecules (3-5 mg of protein). An SH S29A mutant (C) was tested at 20 lipid molecules per SH pentamer (4.8 mg SH). Although the quantity of protein is sufficient, resonances are too broad. This may be due to sample heterogeneity or to intrinsic flexibility in the protein. To improve resolution, lipid composition, protein-to-lipid ratio, temperature and ligands and other constructs will be tested.



2D-DARR spectra of full length $^{13}C^{15}N$-labeled viroporins in DMPC lipid membranes: SARS-CoV E (A), RSV-SH WT (B) and RSV-SH S29A (C). Spectra were collected in a 600 (A-B) or 500 (C) MHz Jeol NMR spectrometer.

***Deadline: May 19, 2017**
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