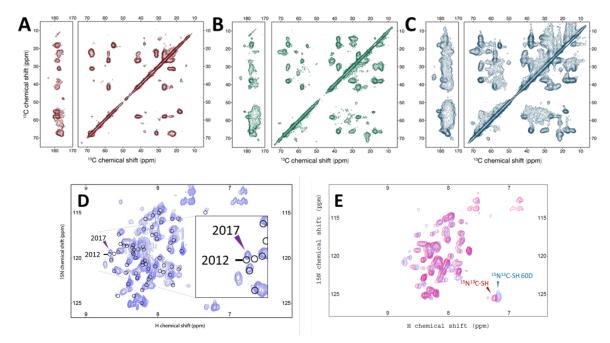
DATE: Day 1 May 2018

SUMMARY of 2017 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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Summary

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. Attempts were made to obtain models of the monomers in lipid membranes using solid-state NMR. For SH protein, we attempted to obtain intermolecular NOEs in micelles by solution NMR. Resonances obtained in solid-state NMR were too broad, probably due to sample heterogeneity or inherent intrinsic flexibility. To improve resolution, high affinity antibodies or ligands are required. Experiments to obtain intermolecular NOEs using a mixed pair (15 N-D and 13 C) in a SH pentameric channel were delayed by shifting resonances and the need to re-assign from scratch, and to insufficient D/H exchange in the deuterated sample.



(A) 2D-DARR spectrum of ¹³C¹⁵N SARS-CoV E protein; (B) RSV-SH protein; (C) RSV-SH protein S29A mutant in DMPC membranes, collected in a 600 MHz Jeol NMR spectrometer; (D) ¹⁵N-Trosy-HSQC of SH in d38-DPC micelles collected in a 800 MHz spectrometer [shifted resonances in previously published data (circles)]; (E) resonance shifts between double labeled SH protein and a partially (60%) deuterated sample.