DATE: Day 25 Month 07 Year 2016

SUMMARY of
2015 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Comprehensive protein dynamics study using NMR spectroscopy
Applicant	**Name**	Rieko Ishima
	Affiliation	University of Pittsburgh School of Medicine
	Present Title	Associate Professor
Research Collaborator (Host PI)		Toshimichi Fujiwara (Professor)

Summary

Since a virus encodes only limited number of genomes, viral proteins often regulate the functions through significant protein conformational changes, which may involve local structural changes, domain folding/unfolding, and dimer or multimer formation. To identify the protein dynamics landscape of such multiple conformational changes, a combination of different methodologies are needed. For this purpose, Ishima group that mainly acquires solution state NMR data has collaborated with Prof. Fujiwara's group that has expertise of solid-state NMR experiments.

Based on try-and-errors on sample preparations for solid-state NMR experiment with enthusiastic support from Dr. Egawa in Fujiwara's group, we could acquire DARR spectrum of a uniformly ^{13}C-labeled protease bound to an inhibitor, darunavir (DRV), to see correlation of the ^{13}C-^{13}C dipolar coupling. ^{13}C chemical shifts observed in the DARR spectrum indicated that the protein mainly forms β-sheet structure. HIV-1 protease is known to contain nine β-strands and one short α-helix in its crystal structure. The DARR data suggests that secondary structure of the protease is mostly maintained in the solid-state NMR sample. Dr. Egawa further supported us to acquire a CHHC spectrum of Ile-methyl labeled sample, and observed weak but significant dipolar couplings, most likely between Ile 85 and Ile 13 and/or between Ile 85 and Ile 66. Although further signal assignments and repeat of experiments at higher protein concentration are needed, the data suggests that the protein core is folded similar to that in solution.

The obtained data in this collaboration provided a fundamental basis for solid-state NMR experiments for HIV-1 protease. Through the collaboration, we found that two NMR techniques, solid and solution state NMR, are getting to be seamless and complementary, making NMR a powerful tool to study chemistry and biology.

***Deadline: July 31, 2016**
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