ELUCIDATION OF DYNAMICS AND ENERGY LANDSCAPE OF MEMBRANE PROTEINS BY SOLID STATE NMR. Daryl Good, Peng Xiao, Philip Drewniak, Dylan Dingwell, Leonid Brown, Rachel Brown, Meaghan Ward, **Vlad Ladizhansky,** Department of Physics and Biophysics Interdepartmental Group, University of Guelph, 50 Stone Road E, Guelph, ON N1G2W1, Canada. (vladizha@uoguelph.ca)

Proteins are dynamic molecules which exist as conformational ensembles. Interconversion between conformational substates occurs either by means of thermal fluctuations or through functionally important motions. The substates were proposed to be organized as a hierarchical energy landscape, with individual energy valleys being separated by barriers whose heights depend on intra-protein interactions as well as on interactions with environment.

Here, we provide examples of how solid-state NMR could be used to probe the energy landscape of membrane proteins. In the first example, we use spin relaxation measurements to probe dynamics of a seven-helical membrane protein proteorhodopsin. We measured temperature dependence of relaxation rates and estimated activation energies of motional modes representing sidechain rotations, local backbone, and sidechain fluctuations as well as higher activation energy collective backbone motions.

In the second example, we use NMR-detected Hydrogen-Deuterium Exchange—to characterize the thermally induced unfolding of a membrane-embedded alpha-helical protein human aquaporin 1 (hAQP1). Its unfolding proceeds through an intermediate state to the misfolded state over two high activation barriers that define the overall protein stability. We show that in hAQP1, folding of loops kinetically stabilizes protein structure, in support of the notion of the third stage in the membrane protein folding model.