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Investigating the Structural Properties of Integral Membrane Proteins with Pulsed EPR Spectroscopy

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Very limited structural and dynamic information on proteins embedded inside a membrane currently exist, because they are difficulty to crystalize. New biophysical/structural biology methods are needed to probe these systems in a lipid bilayer. The Lorigan lab is applying unique hybrid NMR and spin-label EPR spectroscopic techniques to study membrane proteins. Magnetic resonance spectroscopic data of ^{15}N-, ^{2}H-labeled and/or spin-labeled membrane proteins incorporated into vesicles and bicelles will be presented. State-of-the-art pulsed EPR techniques such as Electron Spin Echo Envelope Modulation (ESEEM) spectroscopy, and Double Electron-Electron Resonance (DEER) spectroscopy will be used. The ESEEM technique can determine short to medium range distances (out to about 8 Å) between a site-specific nitroxide spin label and a nearby NMR-active isotopic labeled residue for a variety of different peptides and proteins which ultimately can be used to determine the difference between an α-helical and β-sheet secondary structure. DEER can be used to measure distances between 2 spin labels out to about 70 Å. We have shown a huge improvement is sensitivity with DEER measurements at Q-band when compared to X-band.