Pilot Project Title: Characterization of hydrogen bonding in amphiphilic peptide helices via REDOR using a low-E probe

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Purpose: To obtain NMR data to characterize hydrogen bonding patterns in KL4 when interacting with lipid bilayers. This data will be combined with neutron diffraction data and other ssNMR structural measurements to obtain a high resolution structure of the interaction between KL4 and lipids found in pulmonary surfactant.

Background:
KL4, in combination with phospholipids, can serve as a substitute lung surfactant in premature infants and reduce the incidence of respiratory distress syndrome. How KL4 affects the phase properties of the lipids, the structure and dynamics of the peptide in the lipid membrane, and the molecular level interactions between KL4 with the phospholipids are questions remaining to be answered. The focus of this project is to study the structure, dynamics and partitioning of KL4 in phospholipid membrane preparations to develop a high-resolution molecular model to explain the complex biophysical properties of lung surfactant. The unusual periodicity of hydrophobic and hydrophilic residues in KL4 has led to some controversy regarding both its backbone secondary structure and its orientation in lipid bilayers. Previously, Long et al. have measured peptide backbone torsion angles and peptide sidechain dynamics which have led to a model of KL4 partitioning in the plane of lipid bilayers with an unusual helical structure that places the charged lysine residues on one side of the helix (1-3). This model, combined with molecular dynamics simulations based on NMR constraints, suggests both $i\rightarrow i+4$ and $i\rightarrow i+5$ hydrogen bonding may stabilize the peptide conformation in a lipid bilayer. REDOR measurements would provide a straightforward measurement of the relative populations of these hydrogen bonding patterns at various points in the KL4 helix. These studies will not only answer fundamental questions regarding the interaction between KL4 and lipid bilayers, but will also provide constraints for a comprehensive model of structure-function relationships in lucinactant (the clinical formulation of KL4 with DPPC and POPG) based on a combination of NMR and neutron diffraction measurements.

Figure 1. Intramolecular $^{13}$C'-$^{15}$N distances between leucines in KL4 at positions 8 and 13 from MD simulations using DQDRAWS restraints of the peptide backbone conformation. Separate MD experiments were performed for the three unique sets of NMR constraints.

Research Plan:
Experimental Design: Samples of $^{13}$C-$^{15}$N-labeled KL4 will be prepared with POPC/POPG, and DPPC/POPG lipids. REDOR measurements will be utilized to investigate hydrogen bonding patterns. The low sensitivity of these samples and weak couplings we are measuring make the triple resonance MAS low-E probe critical to the success of this project. Samples will be prepared by the Long lab and sent to FSU for collection and analysis of NMR data by Long, Mehta, and Hung (Figure 2).

Figure 2. $^{13}$C-$^{15}$N REDOR measurements on KL4 $^{13}$C-enriched at L8 and $^{15}$N-enriched at L13 in DPPC:POPG vesicles. (A) Full-echo (bottom) and REDOR difference (top) spectra for a 38.4 ms REDOR evolution time showing dephasing of both the 172 and 176 ppm peaks. (B) $^{13}$C-$^{15}$N REDOR dephasing for signals at 172 ppm (green circles) and 176 ppm (black squares). The solid lines represent calculated dephasing curves for distance distributions obtained in the MD simulations (Fig. 1) for the $\alpha$-helix (red), 172 ppm (green) and 176 ppm (black) ensembles of structures. Dashed lines are calculated REDOR curves for the distributions determined with Boltzmann – Statistics Maximum – Entropy (BS-REDOR) analysis.

NMR: 600 MHz NMR spectrometer with a 3.2 MAS triple resonance low-E probe. REDOR NMR spectra on at least 4 samples