**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 $KL_4$ 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 $KL_4$ and lipids found in pulmonary surfactant.

**Background:**
KLLLLKLLLLKLLLLKLLLLK ($KL_4$), in combination with phospholipids, can serve as a substitute lung surfactant in premature infants and reduce the incidence of respiratory distress syndrome. How $KL_4$ affects the phase properties of the lipids, the structure and dynamics of the peptide in the lipid membrane, and the molecular level interactions between $KL_4$ with the phospholipids are questions remaining to be answered. The focus of this project is to study the structure, dynamics and partitioning of $KL_4$ 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 $KL_4$ has lead 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 $KL_4$ 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→i+4 and i→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 $KL_4$ helix. These studies will not only answer fundamental questions regarding the interaction between $KL_4$ and lipid bilayers, but will also provide constraints for a comprehensive model of structure-function relationships in lucinactant (the clinical formulation of $KL_4$ with DPPC and POPG) based on a combination of NMR and neutron diffraction measurements.

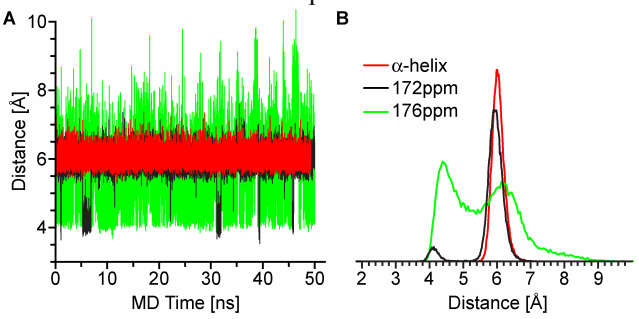


**Figure 1.** Intramolecular $^{13}C'$-$^{15}N$ distances between leucines in $KL_4$ at positions 8 and 13 from MD simulations using DQDRAWS restraints of the peptide backbone conformation. Separate MD experiments were performed for the three unique sets of NMR constraints.

**Research Plan:**
**Experimental Design:** Samples of $^{13}C\{^{15}N\}$-labeled $KL_4$ 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).

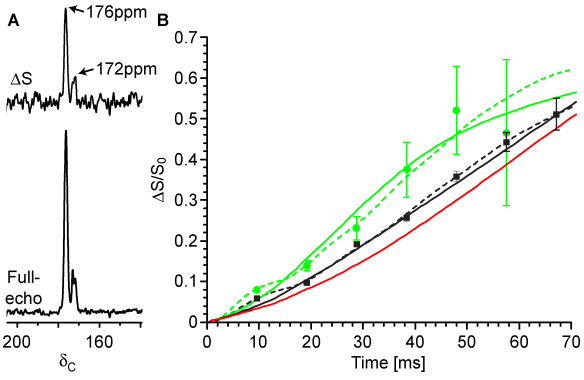


**Figure 2.** $^{13}C\{^{15}N\}$REDOR measurements on $KL_4$ $^{13}C'$-enriched at L8 and $^{15}N$-enriched at L13 in DPPC:POPG vesicles. (A) Full-echo (bottom) and REDOR difference (top) spectra for a 38.4 ms REDOR evolution time showing dephasing of both the 172 and 176 ppm peaks. (B) $^{13}C\{^{15}N\}$ 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 α-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

1. Long, J. R., F. D. Mills, O. K. Ganesh, V. C. Antharam, and R. S. Farver. 2010. Partitioning, dynamics, and orientation of lung surfactant peptide $KL_4$ in phospholipid bilayers. *Biochim Biophys Acta* 1798:216-222.
2. Antharam, V. C., D. W. Elliott, F. D. Mills, R. S. Farver, E. Sternin, and J. R. Long. 2009. Penetration depth of surfactant peptide $KL_4$ into membranes is determined by fatty acid saturation. *Biophys J* 96:4085-4098.
3. Mills, F. D., V. C. Antharam, D. W. Elliott, S. A. McNeill, and J. R. Long. 2008. The helical structure of surfactant peptide $KL_4$ when bound to POPC:POPG lipid vesicles. *Biochemistry* 47: 8292-8300.