

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

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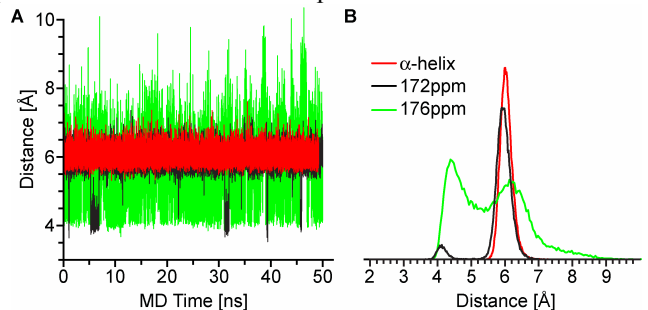
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Purpose: To obtain NMR data to characterize hydrogen bonding patterns in KL₄ when interacting with lipid bilayers. This data will be combined with neutron diffraction data and other ssNMR structural measurements to obtain a high resolution structure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

Background:

KL₄ (KLLLLKLLLLKLLLLKLLLLK), 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*→*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 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 ¹³C-¹⁵N 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 ¹³C{¹⁵N}-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).

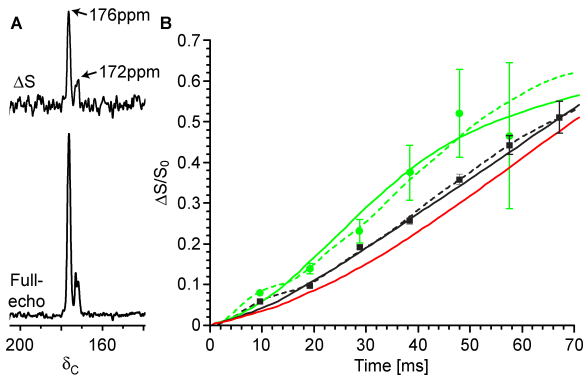


Figure 2. ¹³C{¹⁵N}REDOR measurements on KL₄ ¹³C-enriched at L8 and ¹⁵N-enriched at L13 in DPPC:POPG vesicles. (A) Full-echo (bottom) and REDOR difference (top) spectra for a 38.4 ms REDOR evolution time showing dephasing of both the 172 and 176 ppm peaks. (B) ¹³C{¹⁵N}REDOR dephasing for signals at 172 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 KL₄ 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 KL₄ 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 KL₄ when bound to POPC:POPG lipid vesicles. *Biochemistry* 47: 8292-8300.

Biographical Sketch (Template)

Name:

Title:

Affiliation:

(a) Professional Preparation

A list of the individual's undergraduate and graduate education and postdoctoral training as indicated below:

Undergraduate Institution(s)	Major	Degree & Year
Graduate Institution(s)	Major	Degree & Year
Postdoctoral Institution(s)	Area	Inclusive Dates (years)

(b) Appointments

A list, in reverse chronological order, of all the individual's academic/professional appointments beginning with the current appointment.

(c) Products

A list of: (i) up to five products most closely related to the proposed project; and (ii) up to five other significant products, whether or not related to the proposed project. Acceptable products must be citable and accessible including but not limited to publications, data sets, software, patents, and copyrights. Unacceptable products are unpublished documents not yet submitted for publication, invited lectures, and additional lists of products. Only the list of 10 will be used in the review of the proposal.

Each product must include full citation information including (where applicable and practicable) names of all authors, date of publication or release, title, title of enclosing work such as journal or book, volume, issue, pages, website and Uniform Resource Locator (URL) or other Persistent Identifier.

(d) Synergistic Activities and Accomplishments

A list of up to five examples that demonstrate the broader impact of the individual's professional and scholarly activities that focuses on the integration and transfer of knowledge as well as its creation. Examples could include, among others: innovations in teaching and training (e.g., development of curricular materials and pedagogical methods); contributions to the science of learning; development and/or refinement of research tools; computation methodologies, and algorithms for problem-solving; development of databases to support research and education; broadening the participation of groups underrepresented in science, mathematics, engineering and technology; and service to the scientific and engineering community outside of the individual's immediate organization.