Elucidating the Lipid Fusion Mechanism of Surfactant Protein B_{1-25} with Solution and Solid State NMR

N.T. Tran,[1] *Gail E. Fanucci,*[1] *Joanna R. Long.*[2]

University of Florida, Department of Chemistry, Gainesville, FL[1]

University of Florida, Department of Biochemistry and Molecular Biology, Gainesville, FL[2]

We utilize solution and solid state NMR to characterize lipid dynamics and morphologies in pulmonary surfactant (PS) model lipid systems containing therapeutic levels of surfactant protein B_{1-25} (SP-B_{1-25}). Pulmonary surfactant (PS) is a lipid/protein mixture that resides in the alveolar aqueous environment and is required for proper lung function. Its primary role involves surface tension reduction at the alveolar air-water interface. Of the four surfactant proteins, SP-B is the only protein that promotes trafficking of PS lipids from the alveolar aqueous reservoir to the air-water interface, and thus renders SP-B the only PS protein required for survival. Our ^{2}H and ^{31}P solid state NMR results reveal SP-B_{1-25} induces non-lamellar lipid phase morphology in hydrated assemblies of 4:1 DPPC/POPG at physiologic temperature (37 °C) [1]. ^{31}P T_2 relaxation times confirm this isotropic phase to be consistent with a lipid cubic phase and elucidates the architectural framework arranged by SP-B_{1-25} to allow rapid lipid transit between lamellae. Our variable temperature ^{2}H NMR results indicate SP-B_{1-25} promotes thermal stability of the cubic phase through lipid interdigitation. The coexisting cubic and interdigitated phase is isolated to DPPC lipids and we propose a unique role for DPPC in stabilizing energetics of SP-B_{1-25} induced lipid polymorphisms. Furthermore, we aim to characterize the structure, membrane partitioning and dynamics of SP-B_{1-25} utilizing solution NMR. Our initial solution NMR studies focus on the membrane anchoring N-terminal 7 amino acids (SP-B_{1-7}). Surface tension reduction is not observed in the absence of SP-B_{1-7}, thus structural and dynamic studies of this region is greatly warranted. We hypothesize SP-B_{1-7} to be highly mobile given the presence of three proline residues that can undergo cis-trans isomerization. With solution and solid state NMR, we aim to characterize molecular mechanisms of SP-B_{1-25} induced lipid polymorphisms. Our overall objective is to highlight protein structural and dynamic motifs involved in membrane fusion events.

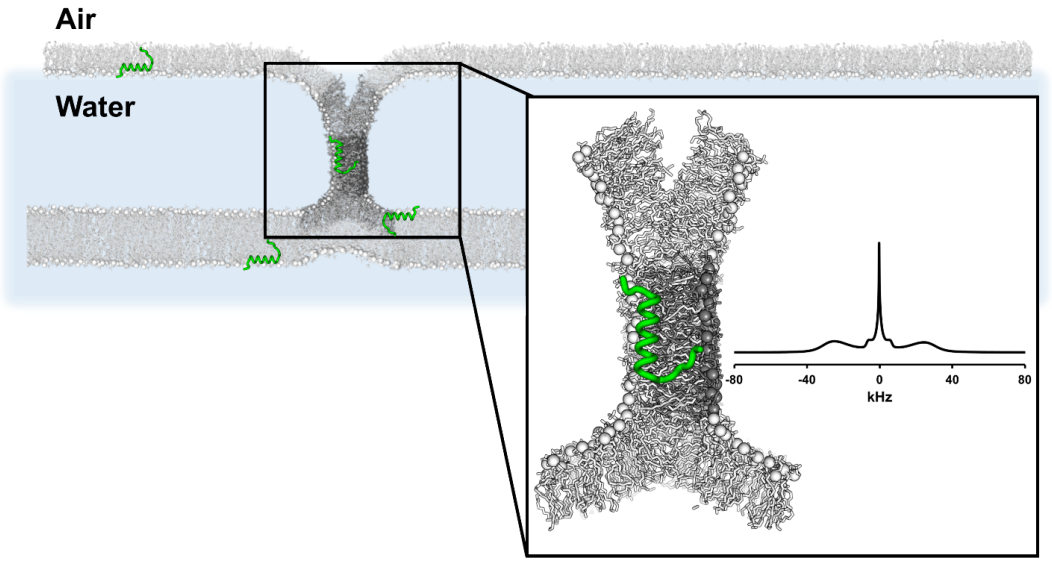

Figure 1. Model of SP-B_{1-25} induced lipid trafficking from the alveolar aqueous reservoir to the air-water interface. (box) Deuterium NMR spectra of 4:1 DPPC-d_{62}/POPG containing 5 mol% SP-B_{1-25} at 37 °C. DPPC is shown to adopt a cubic and interdigitated lipid phase [1].

1. Tran, N. Entropic Anomaly Observed in Lipid Polymorphisms Induced by Surfactant Peptide SP-B(1−25). *J.Phys.Chem.B* **2017**, 121 (39), 9102–9112