# Location of an α-tocopherol in Bilayers of Differing Head Group Composition

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Vitamin E is the only essential vitamin for which it is not known why it is essential. Discovered in 1922 by Bishop et al.,[1] it was observed that without this dietary component rats could no longer reproduce. Vitamin E is composed of two families of molecules known as tocopherols and tocotrienols, each with four members α, β, δ, and γ, which refer to particular substitutions on the chromanol ring. Although the eight components of Vitamin E share many similarities and are all consumed in most diets, α-tocopherol is the only component taken up by the human body.[2,3]

To explain and predict the action of α-tocopherol in membranes, we examined how the phospholipid environment affects the orientation and dynamics of α-tocopherol within the bilayer using neutron diffraction. Membrane bound molecules are not only sensitive to the phospholipid tail composition, but are sensitive to the headgroup and lipid backbone composition as well. Phospholipid headgroups of varying size and charge were studied as well as a ceramide backbone (sphingomylin). POPE was studied as the small phospholipid headgroup and POPS was examined as the charged phospholipid headgroup. POPC acted as the control for comparison with phospholipid tail composition. Past experiments have used PC phospholipids to study the effect the degree of unsaturation the acyl chain has on the location of Vitamin E in a membrane. In addition, POPC also served as a large headgroup to be examined.

Neutron diffraction data were collected at the Canadian Neutron Beam Centre's N5 and D3 beamlines using 2.37 Å wavelength neutrons. The appropriate wavelength neutrons were selected by a pyrolytic graphite (PG) monochromatic, while a PG filter was used to eliminate higher order reflections. Samples were placed in an airtight sample cell (constructed at Brock University), then purged with argon and hydrated to 93% relative humidity (RH) using a series of $D_2O/H_2O$ mixtures (i.e., {100%, 70%, 40% and 8% $D_2O$). RH was controlled by saturating the $D_2O/H_2O$ solutions with $KNO_3$.

The location and distribution of a specific deuterium label on the tocopherol molecule was determined using labeled and unlabeled samples. The C5 methyl of α-tocopherol is highly localized in all cases, which is seen by the very narrow distribution of the label. It was determined that α-tocopherol resides higher in the bilayers with zwitterionic headgroups than with the charged. We observe the C5 label above the hydrogen belt closer to the phospho-headroups for POPC, and POPE. This suggests that α-tocopherol's location in the lipid environment is independent of headgroup size. For the charged POPS and the ceramide backboned sphingomylin, the label is deeper in the bilayer near the backbone, possibly due to an aversion to the charged headgroup. α-tocopherol sitting low in sphingomylin could be due to the tightly packed nature of sphygo-lipids pushing tocopherol into the bilayer. Figure 1 illustrates SLD profiles for the four lipids studied, the water distribution, and the label distribution.

The high location in zwitterionic headgroups supports our hypothesis that α-tocopherol acts as an antioxidant against water borne reactive oxygen species. In the case of POPS and sphingomylin, α-tocopherol still resides high enough in the bilayer to come in contact with a significant amount of the bulk water phase.

**References**

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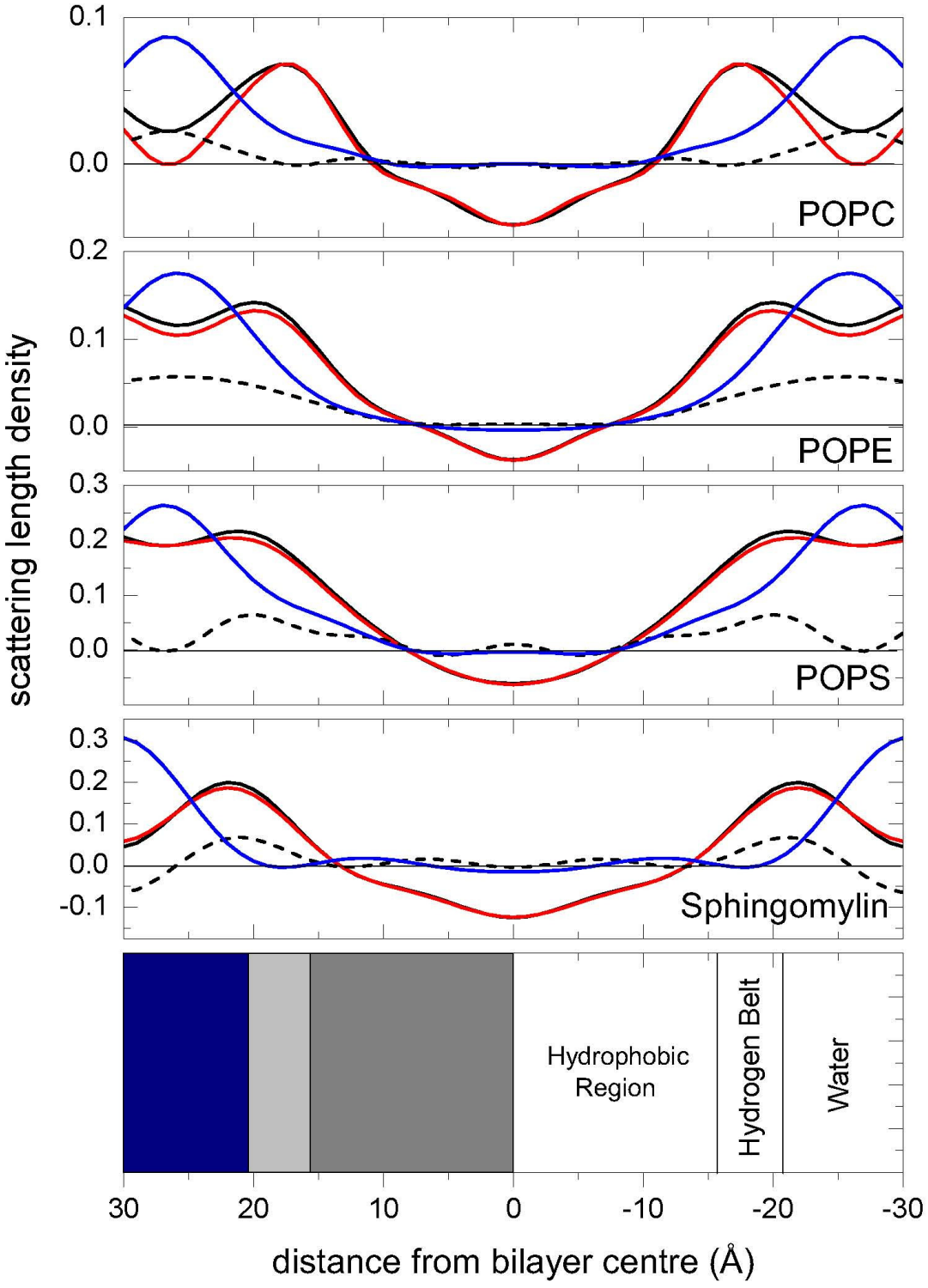


**Fig. 1** The scaled scattering length density (SLD) profiles for labeled (black) and unlabeled (red) α-tocopherol in POPC, POPE, POPS and sphingomylin bilayers. The difference between labeled and unlabeled SLD is the label distribution (dashed line). The water distribution is shown in blue.