# Orientation of α Tocopherol in Saturated Membranes

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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 Bishope 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 4 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 explore how the phospholipid environment affects the orientation and dynamics of α-tocopherol within the bilayer using neutrons.

We used N5/D3 with 2.37 Å wavelength neutrons and with a fixed beam width of 4 mm. High order reflections (λ/2, λ/3 ... ect.) were eliminated by PG filters. Samples were held in a special humidity chamber in nitrogen atmosphere to prevent the oxidation of vitamin E. Data were collected for systems in liquid-cryctalline phase (i.e. 50 °C for di16:0-PC while 25 °C for other lipids). Samples were hydrated using a saturated salt solution of $KNO_3$ (94% RH), each sample was hydrated with 70, 40, 8 and 0 % $D_2O$. We typically collected 4-6 diffraction orders, indicative of well aligned multilayers, Fig 1. The data were corrected and analyzed as outlined previously by Kučerka et. al..[4] After the Fourier reconstruction, the neutron scattering length density was placed on a relative scale, thus we could ascertain where the tocopherol hydroxyl was located.

Neutron diffraction allowed for the elucidation of vitamin E's location in a variety of phospholipids. It was observed that vitamin E resides in 3 locations with no correlation to the chain length or degree of un-saturation present, Fig 2. However, with the aid of UV/Vis spectroscopy we show evidence of an anti-oxidant mechanism of vitamin E which correlates strongly with its physical location of vitamin E in a model lipid bilayer, a mechanism that would remain overlooked without the use of neutron to locate vitamin E position. UV spectroscopy suggests that lipid radical reduction occurs specifically at the hydrophobic-hydrophilic interface of the membrane.

The result is a clear example of the importance of lipid diversity in controlling the dynamic structural properties of biological membranes; i.e. the controlled response to the membrane's susceptibility to oxidization. By testing vitamin E's location in phosphatidylcholine lipids with increasing degrees of acyl-chain unsaturation, we report a biophysical picture of how the oxidation protection of lipids by α-tocopherol is related to its depth in the lipid bilayer.

**References**

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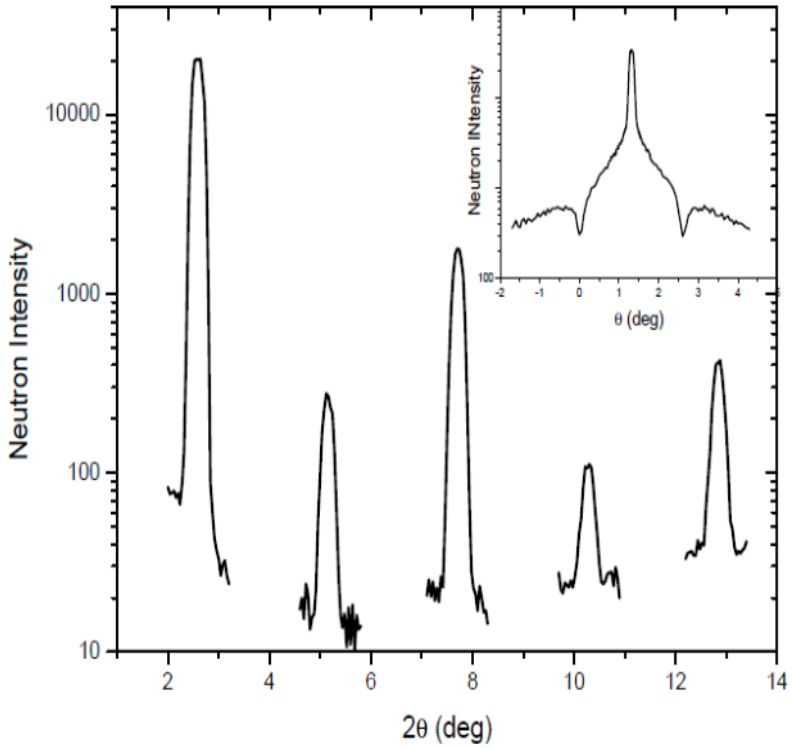


**Figure 1** The main plot is 14:0-14:0 PC with 10 mol% α-tocopherol, hydrated with 40% D2O solution. The inset is the rocking curve, where the detector is at fixed angle and the sample angle is rocked, to characterize the orientation quality of the sample.

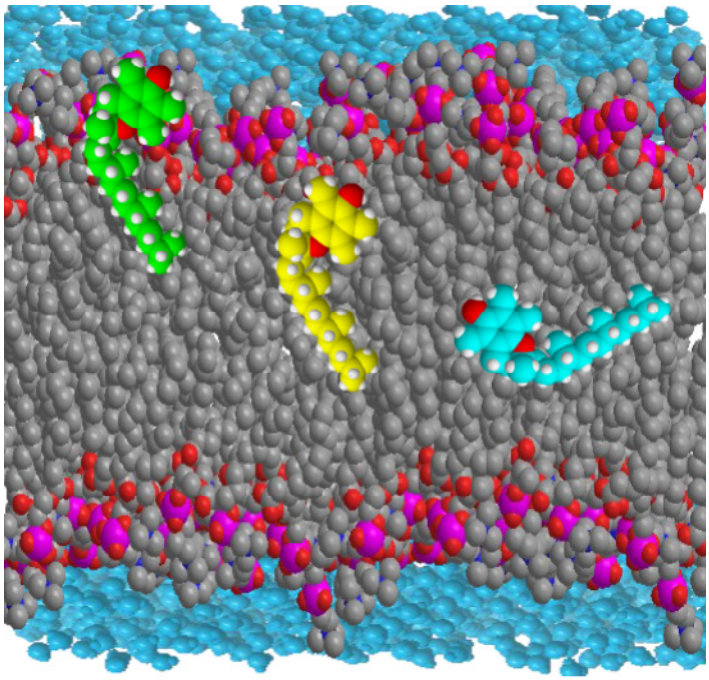

**Figure 2** A schematic representation of the locations that α-tocopherol assumes in different phospholipid bilayers. The green α-tocopherol represents the location of the vitamin in di16:0-PC, 16:0-18:1-PC, and PUFA (di20:4-PC) bilayers, in the vicinity of the PC headgroups. The yellow α-tocopherol represents the location of the vitamin in di 18:1-PC and 16:0-20:4-PC bilayers. In the latter bilayers α-tocopherol resides near the lipid-water interface (i.e. glycerol backbone). The cyan α-tocopherol shows the molecule in the center of di14:0-PC bilayers.