

Macroscopic Alignment and Leaflet Solvent Accessibility of Nanopore-confined Lipid Bilayer Arrays as a Function of Lipid Composition

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Recently, we described a novel method for studying membrane proteins in a native lipid bilayer environment by solid-state NMR spectroscopy. We have shown that nanotubular bilayers formed inside macroscopically aligned nanochannels of anodic aluminum oxide membranes (AAO) retain many biophysical properties of unsupported bilayers and are suitable for aligning membrane proteins for high resolution solid state NMR studies. This method of macroscopic lipid alignment has been found by us as well as by other groups to be useful in studying lipid bilayers and membrane peptides.

Lipid nanotube arrays offer two principal advantages for studying membrane proteins by solid state NMR: 1) the structures retain high hydration level and macroscopic alignment under a broad range of pH and salt concentrations and 2) both leaflets of DMPC lipid nanotubes were accessible to small water soluble molecules allowing for structure-function membrane protein studies by NMR.

Further development of the lipid nanotube arrays as an NMR lipid bilayer alignment method calls for diversity in the bilayer composition that would closely mimic cellular membranes. Here, we report on an NMR investigation of macroscopic alignment and bilayer leaflet surface accessibility for lipid nanotube arrays formed from zwitterionic and anionic lipids of various chain lengths and saturation. In summary, we have succeeded in forming macroscopically aligned lipid bilayers using many phospholipids including anionic lipids and bilayers containing cholesterol. We have also observed ³¹P resonances from these samples under a variety of conditions by varying pH, solution

ionic strength, and exposing samples to a series of paramagnetic ions. Unexpectedly, the leaflet surface accessibility showed a dependence upon the acyl chain length. Specifically, in accord to previously reported MAS NMR by Gawrisch and coworkers the inner leaflets of POPC were found to be inaccessible to metal ions. In contrast, nanotubular bilayers formed from shorter lipids exhibited full ion accessibility based on our ³¹P NMR experiments carried out with macroscopically aligned lipid nanotubes at approximately room temperature (Fig. 2). Variable temperature NMR experiments confirmed that this difference in accessibility properties is attributed not to the phase state of the lipids as previously thought, but rather to the acyl chain length that affects the lipid structures formed in the nanopores.

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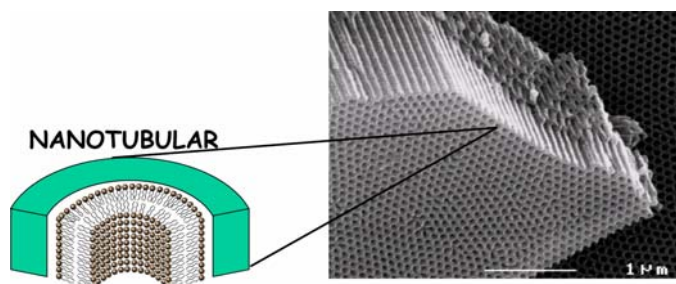


Figure 1. A cartoon of a lipid bilayer confined inside a nanopore and an SEM image of an AAO wafer (right).

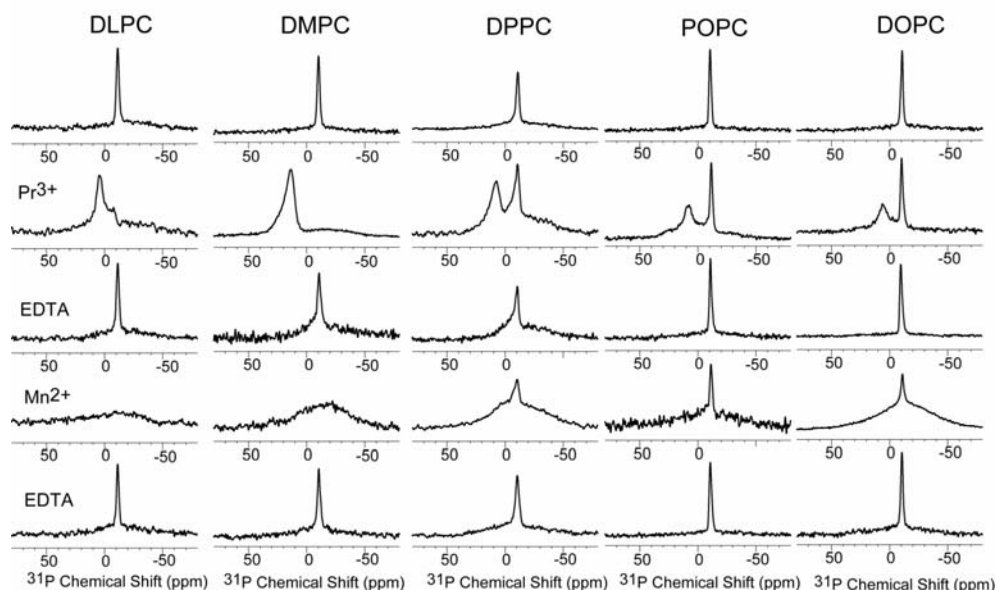


Figure 2. A matrix of ³¹P proton-decoupled 7 T NMR spectra of a series of phospholipids of various chain lengths and different head groups (labeled on the top) confined to the nanoporous support with the average pore diameter of 175 nm. The rows show the spectra after buffer exchange with the buffer type given on the right. Notably, for all samples the ³¹P signal was recovered with a subsequent 50 mM dipotassium EDTA wash. ³¹P chemical shift of 85% H₃PO₄ was referenced as 0 ppm. All samples were kept at 40°C with exception of DPPC (50°C).