

Flow-through Lipid Nanotube Arrays for Macroscopic Alignment and Structure-function Studies of Membrane proteins by Solid-state NMR Spectroscopy

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Solid-state NMR studies of membrane proteins in native cellular environment are hardly ever practical and even those in lipid bilayers are rare. The main reasons for that are technical difficulties of preparing and maintaining membrane protein samples that are both macroscopically aligned and unperturbed by the alignment procedure. It is even more difficult to carry out structure-function studies of membrane proteins that respond to various stimuli or conduct numerous functional activities in lipid bilayers. Here, we report on substrate-supported lipid bilayer methodology that enables such studies by providing (i) macroscopic alignment of lipid bilayers with essentially unperturbed properties and (ii) solvent accessibility to the surfaces of both bilayer leaflets.

Specifically, we employed nanoporous anodic aluminum oxide (AAO) substrates to form macroscopically aligned peptide-containing lipid bilayers that are fluid and highly hydrated. These nanopore-confined bilayers provide an attractive way for aligning membrane protein samples for solid-state NMR experiments. Figure 1 shows a cartoon of a single lipid bilayer aligned inside a nanopore. We demonstrate that surfaces of both leaflets of these nanotubular bilayers are fully accessible to water-soluble molecules, and that the high hydration levels of these structures as well as pH and desirable ion and/or drug concentrations can be easily maintained. We explore these unique features that permit solvent flow through lipid nanotube arrays for structure-function studies of membrane proteins by solid-state NMR spectroscopy. Here, we present a demonstration of such experiments at high magnetic field (19.6 T) using ¹⁷O NMR anisotropic chemical shift effects of ion binding to the gramicidin A (gA) channel.

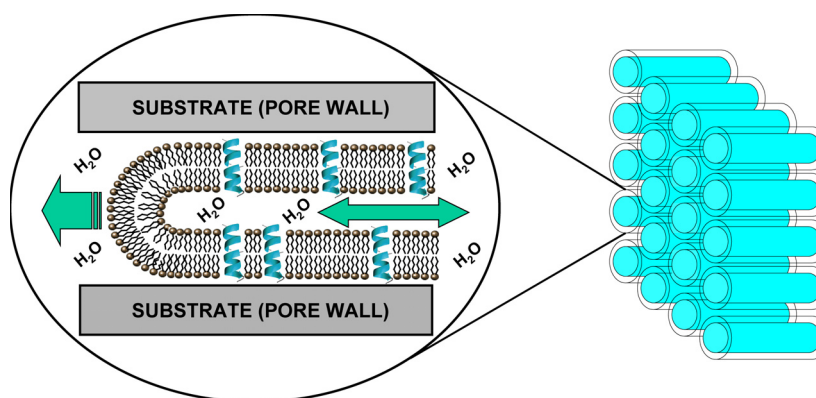


Figure 1. A cartoon of a single lipid bilayer aligned inside a nanopore as a multilamellar aqueous dispersion of phospholipids is drawn inside the pore by capillary action. Arrayed structure of nanoporous channels of AAO substrate is illustrated on the right.

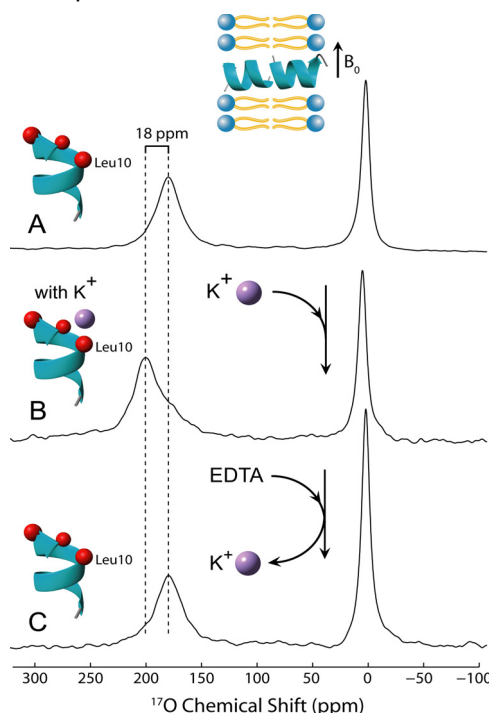


Figure 2. ¹⁷O NMR spectra of ¹⁷O-[D-Leu10]-gA uniformly aligned in DMPC bilayers in the absence and presence of KCl (2.4 M) with a peptide:lipid ratio of 1:16 and excess hydration in AAO-aligned nanotubular bilayers. Spectra were acquired in ~4 hours at 40 °C using an NHMFL static probe at 19.6 T. The lipid bilayer sample contained ~2 μmol of ¹⁷O-[D-Leu10]-gA and was aligned with the magnetic field parallel to the bilayer surface. Note, that the 0 ppm peak is due to the natural abundance ¹⁷O water signal.

The Leu10 carbonyl in the gA pore is one out of three carbonyl sites involved in K⁺ binding. We have monitored K⁺ binding using the ¹⁷O resonance of selectively labeled Leu10 carbonyl when gA dimers were incorporated into DMPC bilayers and aligned using the AAO method. Since the surfaces of nanotubular bilayers are fully accessible to water soluble molecules, we were able to study reversible binding effects of K⁺ to the gA channel from the ¹⁷O chemical shift using the same sample without losing its macroscopic alignment. When the sample was exposed to 2.4 M KCl to generate double occupancy of K⁺ in the dimeric channel, or one ion per monomer, a downfield shift of 18 ppm is observed (Figure 2B). When K⁺ is removed by an EDTA wash, the resonance returns to its original position (Figure 2C).

Acknowledgements. The experiments were largely conducted at the National High Magnetic Field Laboratory, supported by Cooperative Agreement (DMR-0084173) and the State of Florida. This work was supported by the NSF MCB-0235774. A.S. would like to thank DOE Contract DE-FG02-02ER15354 and NIH 1R01GM072897 for support.