**Introduction**

Solid-state NMR spectroscopy is a powerful structural tool to study membrane proteins. This work demonstrates the first example of a high resolution solid-state $^{15}$N 2D PISEMA NMR spectrum of a transmembrane peptide aligned using hydrated cylindrical lipid bilayers formed inside nanoporous anodic aluminum oxide (AAO) substrates.

**Experimental**

The $^{15}$N-Ala-30 M2-TMD was prepared by solid-phase peptide synthesis and co-dissolved in trifluoroethanol with DMPC in a 1:16 molar ratio. The solvent was removed by a rotary evaporator followed by drying under vacuum overnight. The lipid film was rehydrated with a 5 mM phosphate buffer at pH=8.0 containing 10 mM amantadine, the antiviral drug, forming multilamellar liposomes containing the M2-TMD in a tetrameric state. The liposomes were then pelleted by ultracentrifugation at 104,000 g. The pellet was collected and homogenized by bath sonication and then deposited on conventional glass and nanoporous substrate.

**Results and Discussion**

Fig. 1 shows a superposition of $^{15}$N 2D PISEMA spectra of single site $^{15}$N-labeled Ala-30 M2-TMD incorporated into DMPC bilayers and aligned with the help of conventional glass slides and nanoporous AAO substrates. Our findings are accepted for publication in the Journal of Magnetic Resonance.

![Figure 1. Three PISEMA spectra of single site $^{15}$N-labeled Ala-30 M2-TMD on a glass support in DMPC bilayers with the glass strip surfaces perpendicular (doublet at $\sigma^{(15)N}$\approx180 ppm) and parallel (solid lines, doublet at $\sigma^{(15)N}$\approx60 ppm) to B$_0$ and a spectrum from an AAO-supported lipid nanotube array sample (32 $t_1$ increments with 1,088 transients each, 20 mg of the M2-TM domain) with the outer substrate surface parallel to B$_0$ (dotted lines, doublet at $\sigma^{(15)N}$\approx60 ppm). Note, that the spectrum from the AAO-sample was obtained at 9.4 T whereas spectra from the glass-supported sample were recorded at 14.1 T.]

**Conclusions**

Nanoporous AAO substrates may offer several advantages for membrane protein alignment in solid-state NMR studies compared to conventional methods. Higher thermal conductivity of aluminum oxide is expected to suppress thermal gradients associated with inhomogeneous radio frequency heating. Moreover, the nanoporous AAO substrate provides excellent accessibility to the bilayer surface for exposure to solute molecules. Such high accessibility achieved through the substrate nanochannel network could facilitate a wide range of structure-function studies of membrane proteins by solid-state NMR.