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Aligning Membrane Proteins and Peptides with Lipid Nanotube Arrays for Structural NMR and EPR Studies. Eduard Y. Chekmenev, Jun Hu, Peter L. Gor'kov, William W. Brey, Timothy A. Cross, The Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory (NHMFL), Tallahassee, FL 32310, USA, Oleg G. Poluektov, Chemistry Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439, USA, Andres Ruuge, Ali M. Alaouie, and Alex I. Smirnov, Department of Chemistry, North Carolina State University, 2620 Yarbrough Drive, Box 8204, Raleigh, NC 27695-8204, USA

In order to achieve adequate spectral resolution for multidimensional NMR, and also, spin-labeling EPR experiments, the membrane protein samples should be uniformly aligned with respect to the magnetic field axis. Typically, such samples of membrane proteins are prepared using aligned lipid bilayers formed on planar solid substrates or by using magnetic forces to align bicelle discs in the external magnetic field. Here we describe the use of nanopore-supported cylindrical lipid bilayers formed inside anodic aluminum oxide (AAO) substrates to align the transmembrane peptides for magnetic resonance studies. Specifically, we report on 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. The transmembrane domain SSDPLVVA(A- ^{15}N)SIIGILHLILWILDRL of M2 protein from influenza A virus was reconstituted in hydrated 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) bilayers that were macroscopically aligned by a conventional micro slide glass support or by the AAO nanoporous substrate. ^{15}N and ^{31}P NMR spectra demonstrate that both the phospholipids and the protein transmembrane domain are uniformly aligned in the nanopores. Similarly, we provide an EPR demonstration of aligning a spin-labeled gramicidin A using lipid nanotubes composed of DMPC. The main advantage of this new alignment method appears in improving and controlling sample hydration. We also demonstrate that the surface of lipid nanotubes is fully accessible to solvent molecules. Such high accessibility achieved through the substrate nanochannel network could facilitate a wide range of structure-function studies of membrane proteins by magnetic resonance methods. Supported by the DOE Contract DE-FG02-02ER15354 to A.I.S. (NCSU) and by NSF MCB-0235774 to T.A.C. The work at Argonne is supported by the DOE Contract W-31-109-Eng-38 (ANL).