Nanopore-supported Flow-through Lipid Nanotube Arrays for Structure-function Studies of Membrane Proteins in Native Bilayer Environment

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Recently we described a novel method for forming substrate-supported macroscopically-aligned lipid bilayers by self-assembling phospholipids into nanotubular structures inside ordered nanochannels of anodic aluminum oxide (AAO) [1]. We have also shown that these nanotubular bilayers retain many properties of unsupported bilayers and are suitable for incorporating transmembrane peptides and small membrane proteins [2]. This method allows for manipulating imbedded membrane proteins by exposing to different substrates, drugs, ions, and other substances and to observe structural changes spectroscopically.

Here we demonstrate the utility of the lipid nanotube arrays by a solid state 19.6 T ^{17}O NMR study of reversible binding effects of mono- and divalent ions on the chemical shift properties of the Leu10 carbonyl oxygen of transmembrane pore-forming peptide gramicidin A (gA). We also show that nanotubular lipid bilayers retain macroscopic alignment under a wide range of temperatures, pH, and solvent ionic strength and that the surfaces of both bilayer leaflets could be made accessible to the solvent.

We also describe a method for incorporating a large electron-transfer membrane protein complex - bacterial reaction center (RC) protein from Rhodobacter sphaeroides - into substrate-supported lipid nanotubes. We describe several incorporation techniques that result in formation of functional proteonanotubes. By using optical absorption spectroscopy we show that ca. 75% of the RCs retained the functional integrity up to 48 days in storage. The light induced EPR spectrum detected within proteonanotobes was found to be identical to that from isolated RC proteins in unsupported proteoliposomes confirming structural integrity and ability for electron transfer. Complementary spin-label EPR experiments indicated macroscopic lipid alignment in the proteonanotubes. The macroscopic lipid and protein alignment in such structures combined with solvent accessibility would assist structure-function studies with a variety of spectroscopic techniques such as NMR, EPR, and optical spectroscopy without protein crystallization.

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[1] *Smirnov, A. I., Poluektov O. G.,* Substrate-Supported Lipid Nanotube Arrays*, J. Am. Chem. Soc*. **125**, 8434-8435 (2003).

[2] *Chekmenev, E. Y., Gor'kov, P.L., Cross, T. A., Alaouie, A. M., Smirnov, A. I.* "Flow-through lipid nanotube arrays for structure-function studies of membrane proteins by solid-state NMR spectroscopy"*, Biophysical J.,* **91**, 3076-3084 (2006).