

## **Gramicidin A Channels in Substrate-Supported Lipid Nanotube Bilayers**

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Recently, we described self-assembly of phospholipids into nanotubular bilayers when placed inside a nanoporous anodic aluminum oxide membranes (AAO). These structures – which we call lipid nanotube arrays – have a high density of the nanoporous channels providing at least a 600-fold gain in the bilayer surface area for the same size as the planar substrate chips. We have also shown that these new substrate-supported bilayers retain many biophysical properties of unsupported bilayers and suitable for aligning membrane proteins for high resolution solid state NMR studies. Because the surfaces of nanotubular bilayers are fully accessible to water soluble molecules, we were able to study reversible effects of binding of mono- and divalent ions on the chemical shift properties of lipid membranes and a transmembrane pore-forming peptide gramicidin A (gA). Specifically, we have characterized cation solvation by the three carbonyl oxygens forming active binding site of gA pore by  $^{17}\text{O}$  NMR at 19.6 T. We compare the  $^{17}\text{O}$  shifts induced by the ion binding to those induced by the binding of the protons in the pH range of more than 13 units and find a significant difference. This points to different mechanisms of ion and proton conduction by the gA pore. This substrate-supported gA-bilayer system was stable over >13 pH units and temperatures as high as 77 °C. Parallel spin-labeling EPR and DSC measurements indicate that in nanotubular bilayers gA forms dimers and has similar effects on bilayer properties as compared with unsupported multilamellar vesicles. This work demonstrates the utility of lipid nanotube arrays for carrying out structure-function studies of membrane proteins with solid state NMR. Supported by the DOE Contract DE-FG02-02ER15354, NIH 1R01 GM072897 to A.I.S and NSF MCB-0235774.