

Applications of Low- E Static Probes to High Frequency Membrane Protein NMR

Peter L. Gor'kov¹, Eduard Y. Chekmenev¹, Myriam Cotten², Gianluigi Veglia³, Jarrod J. Buffy³, Nathaniel Traaseth³, Timothy A. Cross¹, William W. Brey¹

¹National High Magnetic Field Laboratory, Tallahassee, FL; ²Pacific Lutheran University, Tacoma WA; ³University of Minnesota, Minneapolis, MN



Fig. 1. 900 MHz ¹H-X, 500 µL flat coil probe for oriented membrane proteins.

The protein samples studied by solid state biological NMR can be damaged by the energy that they tend to absorb from the RF electric field E of the probe. Probes that suppress as much of the electric field as possible therefore have a great advantage. Several approaches have been proposed to suppress the electric field [1-4]. We have developed large volume low- E flat coil probes as shown in Fig. 1 for oriented samples of membrane protein in which a multi-turn solenoid matching the size and shape of the 500 µL sample forms a sensitive and tunable observe coil. The observe coil is placed within an orthogonal loop gap resonator which produces the ¹H decoupling field with minimal electric field component, thus reducing sample heating. These probes are now routinely used for PISEMA studies of oriented membrane proteins at 900 and 600 MHz (examples in Figs. 2 and 3). At 600 MHz, the RF power dissipated inside the sample has been reduced 5X when compared to a conventional solid-state probe with 4-turn double-tuned solenoid of similar dimensions [5]. Lower electric fields in the sample coil have allowed significant reductions in recovery time, and hence shorter experiments, without dehydration or other damage to the sample, which is especially valuable at 900 MHz. Use of independent single resonance matching networks and orthogonal coils results in efficient probe channels due to the absence of lossy traps required in multi-tuned circuits. The outer resonator provides excellent RF field homogeneity across the sample and wavelength effects need not be accounted for, even for very large samples.

Low- E probes have made it possible to study large samples of aligned membrane protein in our new 900 MHz magnet.

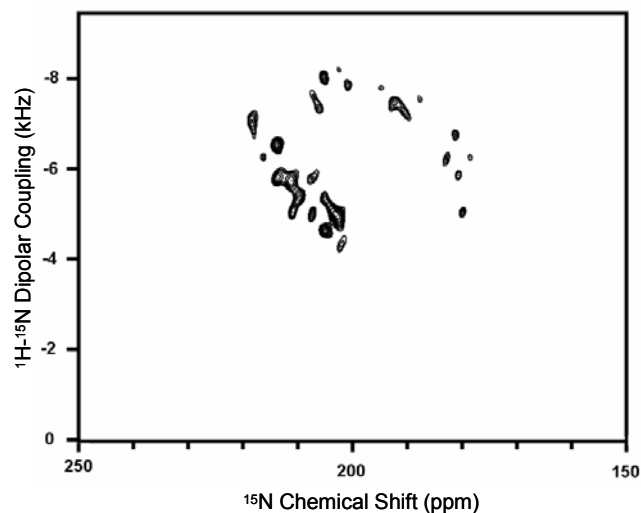


Fig. 2

900 MHz ¹⁵N-¹H PISEMA spectrum of uniformly ¹⁵N-labeled (31 sites) sarcolipin (SLN) membrane protein in oriented lipid bilayers.

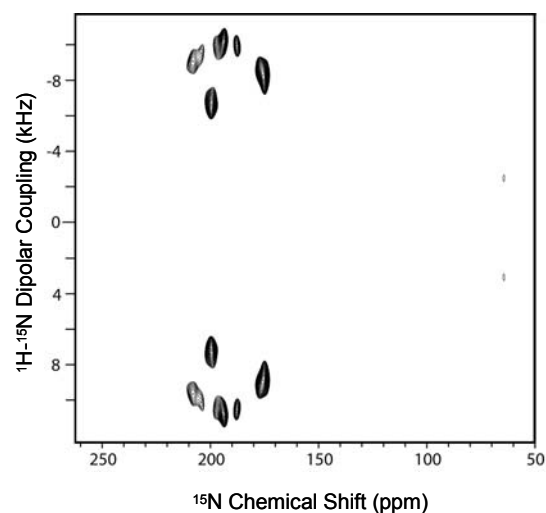


Fig. 3.

600 MHz ¹⁵N-¹H PISEMA spectrum of ¹⁵N Leu (6 sites) labeled 4 kDa trans-membrane protein in oriented lipid bilayers.

References:

1. D.G. Gadian and F.N.H. Robinson, J. of Magn. Reson. **34** (2): 449-455 (1979)
2. D.W. Alderman and D.M. Grant, J. Magn. Reson. **36**, 447-451 (1979)
3. J.A. Stringer, *et al.*, J. of Magn. Reson. **173** (1): 40-48 (2005)
4. K. Pamarthy, *et al.*, 45th ENC (2004)
5. P.L. Gor'kov, *et al.*, 46th ENC (2005)