

## Development of NMR Probe for PISEMA Studies of Membrane Proteins

Peter L. Gor'kov, Kiran K. Shetty, Saikat Saha, Eduard Y. Chekmenev, Jun Hu, Riqiang Fu, William W. Brey

National High Magnetic Field Laboratory, Tallahassee, FL

A balanced RF probe has been developed for studies of large samples of aligned membrane proteins at 600 MHz. Our static coil volume of 600  $\mu\text{L}$  is much larger than an MAS coil volume of  $\sim 130 \mu\text{L}$ , and presents special difficulties. The double resonance probe is designed to carry out polarization inversion spin exchange at the magic angle (PISEMA) experiments to obtain orientational constraints for the proteins in their native lipid environment. Preliminary spectra, such as the pair shown in Figure 1, indicate that the linewidth has been reduced to 700 Hz along the dipolar dimension and 8 ppm along the chemical shift axis by a combination of improving the probe and increasing  $B_0$ .

The matching circuit employs a shorted  $^1\text{H}$   $\lambda/4$  cable to provide a path to ground for the  $^{15}\text{N}$  channel while not greatly disturbing the  $^1\text{H}$  channel. As noted in (1), the stub limits the efficiency of both the  $^{15}\text{N}$  and  $^1\text{H}$  channels. Direct calculation indicated that minimizing the attenuation coefficient ( $\alpha$ ) of the coaxial trap does not give optimal performance in both channels. For the  $^{15}\text{N}$  channel, it appears that efficiency can be improved by increasing the diameter of the inner conductor ( $d_i$ ) beyond the value required to give the minimum  $\alpha$ . Alternately, the dielectric constant ( $\epsilon_r$ ) can be increased to reduce the length ( $L$ ) of the trap. Both approaches reduce the AC series resistance of the trap, and both would be expected to reduce the  $^1\text{H}$  efficiency of the probe. However, because the probe is used for  $^{15}\text{N}$  detection, it was judged that some loss of  $^1\text{H}$  efficiency might be acceptable.

Description	$Z_0$ ( $\Omega$ )	$\epsilon_r$	$d_i$ (mm)	$L$ (mm)	$^1\text{H } t_{90}$ ( $\mu\text{s}$ )	$^{15}\text{N } t_{90}$ ( $\mu\text{s}$ )
UT-390	50	2.0	2.6	83	4.5	5.7
Alumina	11	9.8	4.8	38	7.0	4.2
PTFE	23	2.1	4.8	84	5.6	4.6
Air Line	34	1.0	4.8	120	4.7	4.9

Table 1. The 600 MHz probe was tested with four  $\lambda/4$  coaxial traps all having an outer diameter of 8.4 mm but with varying diameter of the inner conductor ( $d_i$ ) and dielectric constant ( $\epsilon_r$ ) to produce length ( $L$ ) and impedance ( $Z_0$ ). The  $^1\text{H}$  power is 75 W and the  $^{15}\text{N}$  power is 670 W.

To test the efficiency predictions, a 50  $\Omega$  stub made from commercial PTFE-filled coaxial cable (Micro-Coax, Pottstown, PA) was replaced with homebuilt stubs having the same shield diameter but with nearly twice the  $d_i$  and with three different dielectric materials. In order to remain at  $\lambda/4$ , the stubs have varying lengths.  $^1\text{H}$  and  $^{15}\text{N}$  pulse lengths were measured for samples of 100% neutral paraffinic oil and  $(\text{NH}_4)_2\text{SO}_4$ , respectively. The results in Table 1 indicate that the  $^{15}\text{N}$  performance was best with a large  $d_i$  and  $\epsilon_r$ , while  $^1\text{H}$  was best with the highest  $Z_0$ . Of all the traps tested, the air line was judged to give the best combination of  $^1\text{H}$  and  $^{15}\text{N}$  performance for our PISEMA experiments. The air line reduced the  $^{15}\text{N}$  pulse length by 14% at a cost of a 4% increase in the  $^1\text{H}$  pulse length. The air line is now installed in our user probe.

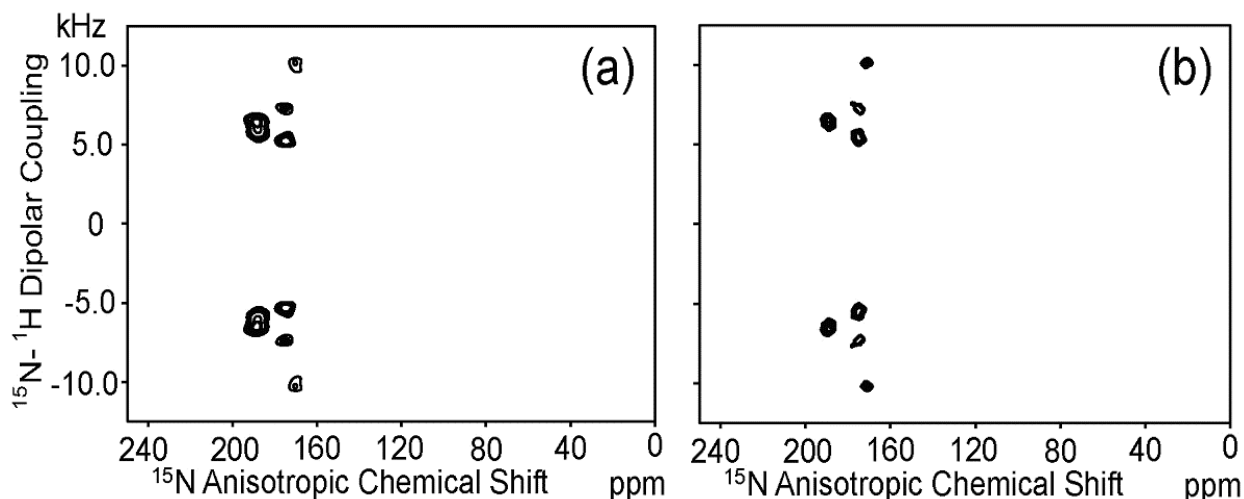


Figure 1. 2D PISEMA spectra of the  $^{15}\text{N}$ -Leu<sub>26,36,38,40,43</sub>-M2-TMP oriented in DMPC in the presence of 10 mM amantadine recorded on (a) 400 MHz with unoptimized, unbalanced probe and (b) 600 MHz. On the 400 system, about 15 mg of the M2-TMP peptide was used for the oriented sample, and 32  $t_1$  increments were used. For each increment, 1024 scans were used to accumulate  $^{15}\text{N}$  signals. On the 600, about 8 mg of the M2-TMP peptide were used. For each increment, 128 scans were used to accumulate  $^{15}\text{N}$  signals. A recycle delay of 6 s was employed in both experiments.

1. F.D. Doty, R.I. Inners, and P.D. Ellis, *J. Magn. Reson.* **43**, 399 (1981).