# AMRIS Update

## Ultra High Sensitivity NMR: 1-mm HTS Triple Resonance Probe

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It seems that every few months the Magnet Lab sets another world record for laboratory magnets. This is always wonderful news, even for those of us who don't use these magnets. It is exciting to imagine the huge fields and stored energy of these systems. At the other end of the scale, however, a four-year collaborative effort between colleagues in AMRIS, the Magnet Lab, and Bruker has yielded a very small world record: a 600 MHz triple resonance 1-mm high temperature superconducting NMR probe that delivers what appears to be the highest mass sensitivity of any probe at any frequency.

The project was funded by an NIH P41 resource grant to UF, but its origins were established over a decade ago in a company called Conductus. Three of us (Bill Brey, Rob Nast and Rich Withers) and others at Conductus developed the first HTS (high temperature superconducting) NMR probes in the early 1990s. In the mid-1990s, Bruker purchased the HTS NMR probe part of Conductus; Withers and Nast continued at Bruker, and Brey moved to the Magnet Lab. Since this project was completed, Withers and Nast have moved from Bruker to Varian, Inc. The overall goal of the NIH resource grant is to improve the sensitivity of NMR and MRI through novel probes and coils, and we therefore formed a new collaboration to develop the world's most sensitive NMR probe. Much of the design and simulation for the probe was done at the Magnet Lab, and the testing and demonstration of capabilities is being done in AMRIS. The project could not have been completed without an industrial partner because it builds upon the commercial Bruker cryoprobe and cryoplatform, and the final product is in a standard cryoprobe body. That is where the similarities to commercial probes end.

In general, the smaller the NMR sample coil the higher the sensitivity; this is the basis of great success in microsolenoid coils (Olson *et al*., 1995; Li *et al*., 2003). It is also possible to cool NMR coils and preamplifiers to reduce noise in measurements; this is the basis of cryogenically cooled probes, most of which are constructed from standard copper wire (Kovacs *et al.*, 2005). Finally, although they are difficult or impossible to fabricate like standard copper wire, HTS materials can provide extremely high sensitivity for NMR measurements (Brey *et al.*, 1996). We combined small sample volume with cryogenic cooling and high-temperature superconducting (HTS) technology to build a 600 MHz 1-mm triple-resonance probe with a z-axis gradient. The probe has an active volume of 6.3 µL and minimum total sample volume of 7.5 µL. The sample is loaded vertically using small capillary tubes. Four nested pairs of resonant HTS coils (Fig. 1) produce frequencies for $^{1}H$, $^{2}H$, $^{13}C$, and $^{15}N$.

All the planar coils are cooled to about 20 K, and the $^{1}H$, $^{2}H$, and $^{13}C$ preamplifiers are cooled to 77 K using a commercial Bruker Cryoplatform. The 1-mm sample chamber is vacuum-insulated from the HTS coils and is warmed by a stream of air for temperature regulation. The $^{1}H$ coil pair (Fig. 2) has a large height-to-width ratio to produce a very homogeneous RF field. To give the best sensitivity, it is placed very close to the sample. Full 3D electromagnetic simulations conducted before the probe was built predicted that the ratio of NMR signal amplitudes following 810° and 90° pulses, a standard measure of B1 homogeneity, would be about 90%. The experimental value is about 87%, in

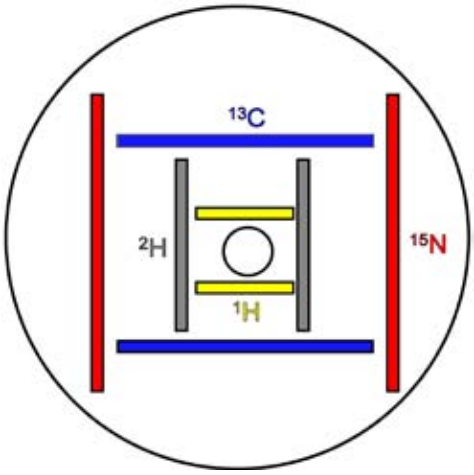


**Figure 1**. Design of the 1-mm HTS triple-resonance probe. Coils were constructed by depositing a thin coating of $Y_1Ba_2Cu_3O_{7-\delta}$ (YBCO) on a sapphire supporting surface.

great agreement with the simulations. The $^{15}N$ coils (Fig. 3) are constructed as spiral resonators to achieve a resonance frequency of 60 MHz. The simple spiral design supports additional unwanted modes at approximate multiples of 60 MHz. Final frequency-trimming relied upon extensive simulations to prevent these modes from interfering with the other coils. The development of accurate simulations for HTS coils was one of the major accomplishments from this research project and will help with new HTS projects in the future.

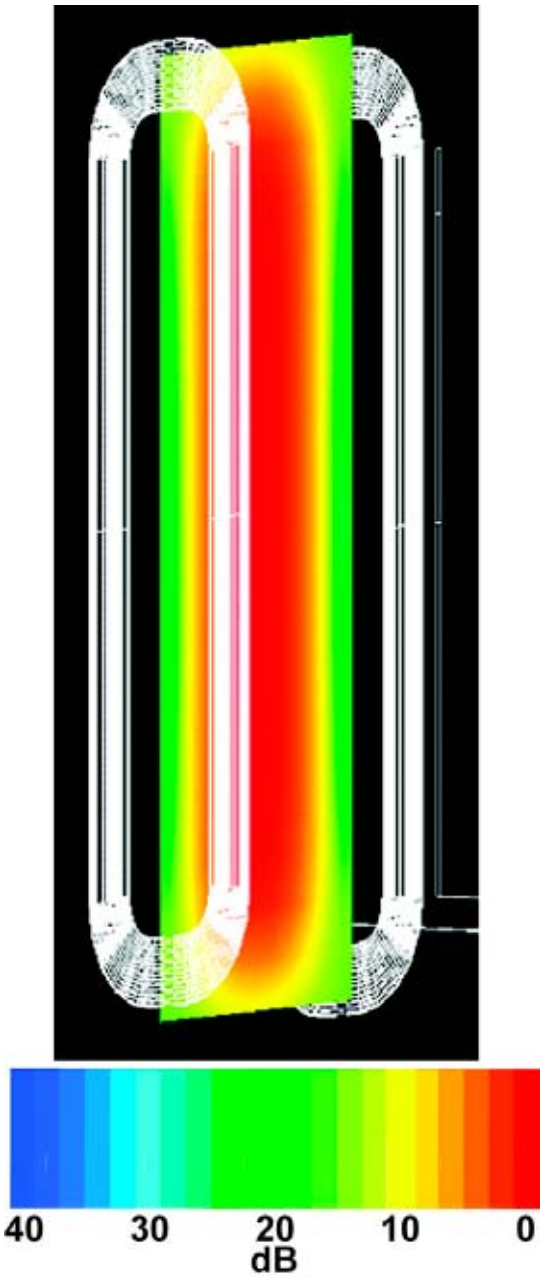


**Figure 2.** Design and simulation of $^{1}H$ coils. The $^{1}H$ coils have a large length to width ratio to optimize $B_1$ homogeneity and active sample volume. Electromagnetic simulations were carried out using IE3D (Zeland Software, Fremont, CA) and predicted excellent $B_1$ homogeneity of 90% for an 810°/90° pulse ratio. This number agrees very well with the experimental value of 86.8%.

The probe was completed the end of summer 2005 and installed in the AMRIS 600 MHz spectrometer in Fall 2005. We have made initial measurements to test the overall performance. One of the most important specifications is the signal-to-noise ratio (S/N). The industry standard for this spec is the height of a certain peak in the spectrum of a solution of 0.1% ethylbenzene in deuterated chloroform following a single 90° pulse. This rather innocent-sounding measurement has many complicated nuances that have been optimized to sell spectrometers, and all users should be aware of some of the potential problems: e.g., the definition of the noise varies rather significantly from vendor to vendor; different "standard" tubes with different wall thicknesses will produce different results. When working with very small sample volumes, we have found additional problems: two different "standard" commercially sealed tubes of 0.1% ethylbenzene differed in our measurements by about 20%. Presumably this is due, in part, to the volatility of the chloroform, a problem that would be amplified with very small volumes. Second, in general it is very difficult with small volume samples to keep them dry, especially in Florida! Therefore, we have found that sample preparation is a very important factor that needs to be carefully planned for optimal performance of the 1-mm HTS probe. Given all of these problems and caveats, we obtained S/N values for 0.1% ethylbenzene in a range from 262 to 364 with an average value of 292±28. This is approximately 3.5-fold less than a standard 600 MHz 5-mm triple-resonance probe (S/N ~1000 with all the caveats) with about 70-fold less sample. Thus, the mass sensitivity of the 1-mm HTS probe is about 20 times greater than a conventional 5-mm probe. Commercial 5-mm Bruker 600 and 800 MHz cryoprobes have S/N

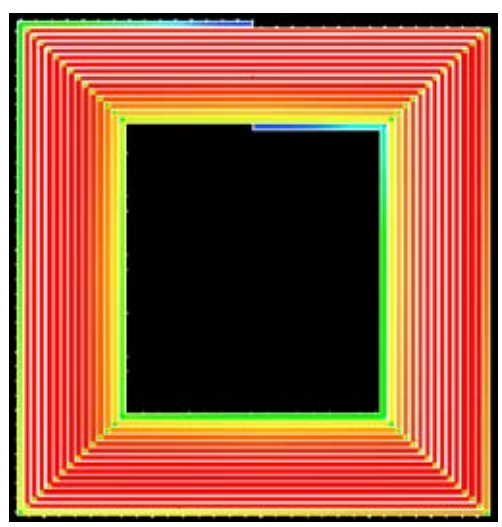

**Figure 3.** Current distribution in the square spiral $^{15}N$ coil. Tuning HTS coils to low frequencies (e.g., 60 MHz for $^{15}N$ at 14.1 T) is challenging and required a new rectangular coil design. The first resonant $^{15}N$ mode is shown, and the red and blue/green colors represent regions with high and low currents, respectively. Extensive computer simulations were required to achieve the correct 60 MHz frequency while simultaneously eliminating parasitic interfering higher order modes, especially 600 MHz.

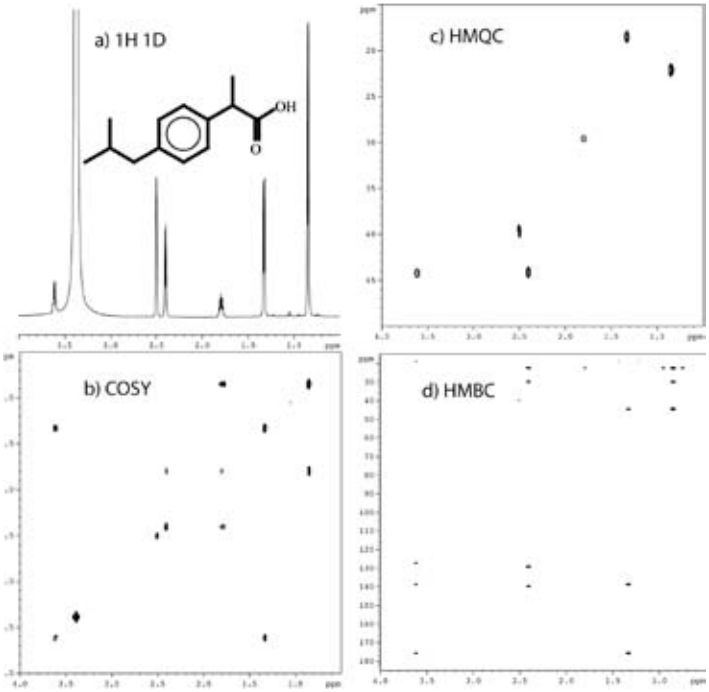


**Figure 4.** Experimental data from the 1-mm HTS triple-resonance probe. Data were collected using 22.6 µg ibuprofen dissolved in 10 µL DMSO-D6 for an ibuprofen concentration of 11.1 mM. (a) 1D spectrum collected with 8 scans, (b) 2D COSY recorded in 3 h 49 m using 4 scans and 1024 $t_1$ increments, (c) $^{13}$C-HMQC recorded with natural abundance $^{13}$C in 16 h using 28 scans and 512 $t_1$ increments, and (d) $^{13}$C-HMBC recorded in 13 h 17 m using 32 scans and 464 $t_1$ increments. We have also collected very good 1D, COSY and HMQC datasets on just 1.7 µg ibuprofen (1.1 mM) (data not shown).

values approximately 4,000 and 8,000, respectively (don't forget the caveats!). The 1-mm HTS probe has a mass sensitivity that is over 4 times greater than a 5-mm cryogenic probe at the same field strength and over 2 times greater than state-of-the-art 5-mm technology at 800 MHz.

How good is the HTS when compared with microsolenoid probes? Unfortunately, these probes are not typically tested with 0.1% ethylbenzene standards, perhaps because the organic solvent could dissolve the seals on the horizontal sample tubes or, for flow systems, damage the flow tubing. The "standard" measurement for these probes tends to be sucrose, but the concentrations used vary quite extensively. We measured the S/N anomeric $^1$H from two different 10.0 mM preparations of sucrose in $D_2O$ using 1, 4, and 8 scans in the HTS probe. Each condition was repeated three times for a total of 18 measurements. These yielded a S/N per µmol of 2338±134 for the active volume (6.3 µl) and 1964±112 for the total volume of sample used (7.5 µl). Comparable measurements in a commercial 1-mm solenoid probe were 2130 for the active volume (1.5 µl) and 639 for the total volume of sample (5 µl) (Olson et al., 2004). Thus, the 1-mm HTS probe has better absolute mass sensitivity and much better total volume sensitivity than a microsolenoid probe at the same field strength.

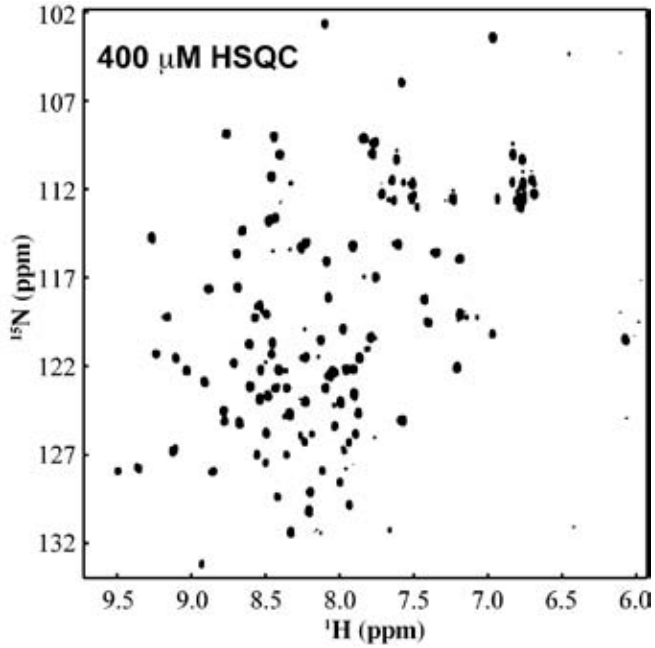


**Figure 5.** Experimental data from the 1-mm HTS triple-resonance probe. Data were collected using 400 µM ubiquitin using gradient $^{15}$N-HSQC and were recorded in 41 m using 16 scans and 128 $t_1$ increments. High quality data on 8 µL 1 mM samples can be obtained in about 10 min (not shown).

The 1-mm HTS probe appears ideal to measure very small masses of sample. If there is ample sample, then larger volume probes will provide greater absolute S/N. However, there are important applications where sample is limited or it would be useful to conserve sample: natural products, metabolomics, and protein screening. We have collected initial experimental results for ibuprofen at natural $^{13}$C isotopic abundance (Fig. 4) and $^{15}$N-labeled ubiquitin (Fig. 5). The ibuprofen spectra (Fig. 4 a-d) were collected with 22.6 µg in 10 µl DMSO-D6 (11 mM). We also successfully collected data on just 1.7 µg in 7.5 µl DMSO-D6 (1.1 mM) (not shown). We have also acquired protein NMR $^{15}$N-HSQC spectra (Fig. 5) in just over 40 min on 400 µM $^{15}$N-labeled ubiquitin in 8 µl phosphate buffer (pH 5.5) with 10% $D_2O$ for lock (Fig. 5).

In conclusion, we have designed, built, and tested a novel NMR probe that appears to have the highest mass sensitivity at any field strength. Although we are still learning many things about optimal use of this probe, it is now part of the Magnet Lab user program in the AMRIS facility, and scientists with mass-limited samples are encouraged to contact Art Edison, the AMRIS Director, *(art@mbi.ufl.edu)* to use this exciting probe for their studies.


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