Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Magnetic Resonance 179 (2006) 290–293
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

Communication

Design, construction, and validation of a 1-mm triple-resonance high-temperature-superconducting probe for NMR

William W. Brey [a], Arthur S. Edison [a,b,c,*], Robert E. Nast [d,1], James R. Rocca [c], Saikat Saha [a,2], Richard S. Withers [d,1]

[a] National High Magnetic Field Laboratory, 1800 E. Paul Dirac Dr., Tallahassee, FL 32310, USA
[b] Box 100245, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610-0245, USA
[c] McKnight Brain Institute, 100 S. Newell Drive, University of Florida, Gainesville, FL 32610-0015, USA
[d] Bruker Biospin, 2859 Bayview Drive, Fremont, CA 94538, USA

Received 29 November 2005; revised 20 December 2005
Available online 19 January 2006

Abstract

We report a 600-MHz 1-mm triple-resonance high-temperature-superconducting (HTS) probe for nuclear magnetic resonance spectroscopy. The probe has a real sample volume of about 7.5 μl, an active volume of 6.3 μl, and appears to have the highest mass sensitivity at any field strength. The probe is constructed with four sets of HTS coils that are tuned to ^{1}H, ^{2}H, ^{13}C, and ^{15}N, and there is a z-axis gradient. The coils are cooled with a conventional Bruker CryoPlatform to about 20 K, and the sample chamber can be regulated above or below room temperature over a moderate range using a Bruker variable temperature unit. The absolute S/N for 0.1% ethylbenzene is approximately 1/3 that of a conventional 5 mm probe with just 1/70 of the sample volume. We demonstrate the utility of this probe for small molecules and proteins with 2D spectra of just 1.7 μg of ibuprofen and 400 μM ^{15}N-labeled ubiquitin.

Keywords: Superconducting probe; Biological NMR; Metabolomics; Protein screening; Natural products; Small volume

1. Introduction

Nuclear magnetic resonance (NMR) is notoriously insensitive due to the very small equilibrium polarization at temperatures appropriate for biological samples and field strengths achievable with superconductive magnets. Signal-to-noise (S/N) can be improved with higher magnetic field strengths, by lowering the temperature of the sample, or by improving the performance of the radio frequency (RF) probe and electronics. RF probes can be improved in three general ways: First, reducing the volume of the detection coil increases the signal per nuclear spin [1], a concept that has been very effectively applied in copper solenoidal microcoils [2,3]. Second, cooling the coils and preamplifiers reduces noise by up to a factor of four [4]. Finally, coils built from low-loss materials such as superconductors improve the sensitivity [5–7].

We combined small sample volume with cryogenic cooling and high-temperature superconducting (HTS) technology [5–7] to build a 600 MHz 1-mm triple-resonance probe with a z-axis gradient (Fig. 1). This probe has an active volume of 6.3 μl and real sample volume of 7.5 μl. The sample is loaded vertically. Four nested Helmholtz pairs of resonant HTS coils (Fig. 1A) produce frequencies for ^{1}H, ^{2}H, ^{13}C, and ^{15}N. All eight HTS coils are cooled to about 20 K, and the ^{1}H, ^{2}H, and ^{13}C preamplifiers are cooled to 77 K using a 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. 1B) has two features that allow it

* Corresponding author. Fax: +1 352 392 3422.
E-mail address: art@mbi.ufl.edu (A.S. Edison).
[1] Present address: Varian, Inc., 3120 Hansen Way, M/S D317 Palo Alto, CA 94304-1030, USA.
[2] Present address: GE Healthcare, Florence, SC 29501, USA.

1090-7807/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2005.12.008

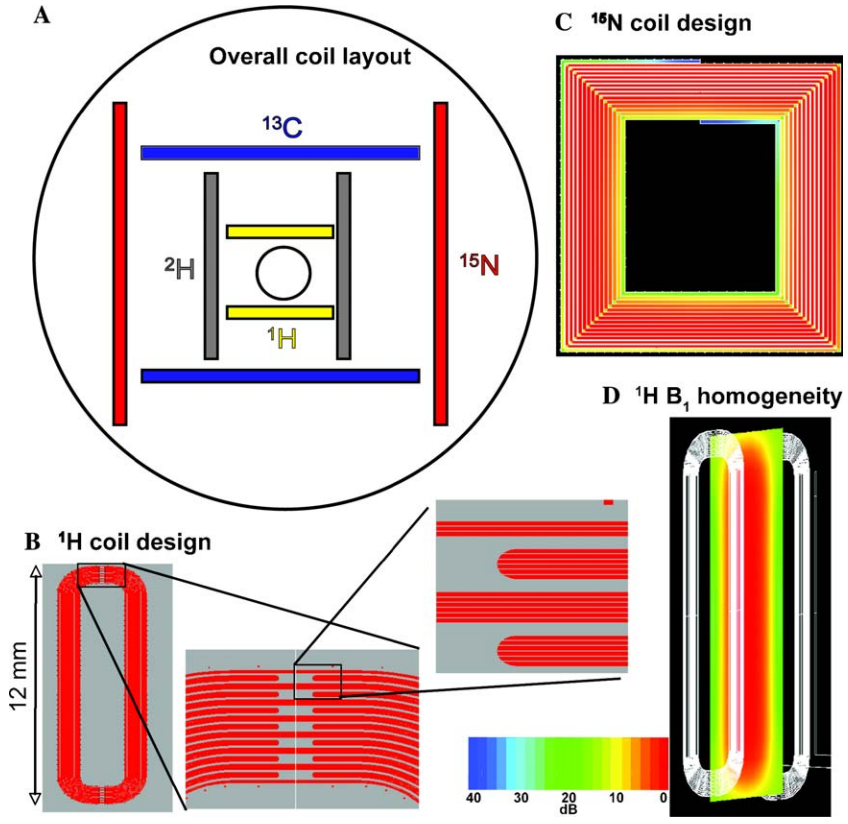

Fig. 1. Design of the 1-mm HTS triple-resonance probe. Coils were constructed by depositing a thin coating of $Y_1Ba_2Cu_3O_{7-\delta}$ (YBCO) on a sapphire supporting surface. (A) Overall coil layout. (B) Details of the interdigitated design of the ^1H coil to create the correct resonance at 600 MHz while minimizing parasitic currents. (C) Current distribution in the square spiral ^{15}N coil. The first resonant ^{15}N mode is shown, and the red and blue/green colors represent regions with high and low currents, respectively. (D) Simulated B_1 homogeneity of the ^1H coil was 90% for an 810°/90° pulse ratio. This number agrees very well with the experimental value of 86.8% (Table 1). Electromagnetic simulations were carried out using IE3D (Zeland Software, Fremont, CA).

to be placed very close to the sample: First, the resonators, based on two distributed interdigital capacitors, with a spatial periodicity of only 0.125 mm, place a very low fringing electric field on the sample. Second, the current-carrying fingers are extensively slit to reduce shielding currents that would reduce B_0 homogeneity [7]. The ^1H coil pair (Fig. 1D) also has a large height-to-width ratio to produce a homogeneous RF field. The ^2H and ^{13}C coils are spiral resonators. Lock sensitivity with such a small sample volume was given higher priority than carbon-observe sensitivity, and for this reason the ^2H coils are in the second position from the inside, and the ^{13}C coils are in the third position. On the outside are the ^{15}N coils (Fig. 1C), also spiral resonators to achieve a resonance frequency of 60 MHz. Because the ^{15}N coils are far from the sample, their rather large electric field is acceptable. However, the simple spiral design supports additional modes at approximate multiples of 60 MHz. Final frequency-trimming relied upon extensive simulations to prevent these modes from interfering with the other coils.

Experimental performance of the probe is listed in Table 1. There is good agreement between the simulated (Fig. 1D) and experimental ^1H B_1 homogeneity, validating the simulations. Similar agreements were found in several

Table 1
Measurements of 1-mm HTS probe with standard samples

	^1H	^{13}C	^{15}N	^2H
90° Pulse length @ power	9.5 μs @ 1.0 W	15 μs @ 18.5 W	48 μs @ 9.5 W	260 μs @ 80 mW
B_1 homogeneity	90.8% (450/90)	83.8% (360/0)	90.8% (360/0)	ND
	86.8% (810/90)	80.6% (720/0)	65.4% (720/0)	
S/N	292 ± 28[a]	39[b]	ND	ND
Lineshape[c]	Spinning: 1.05/9.6/15.2 Hz	ND	ND	ND
	Non-spinning: 0.88/13.9/20.9 Hz			

[a] Four measurements each on two different factory sealed 0.1% ethylbenzene, $CDCl_3$ standards.
[b] 40% dioxane/C6D6 (ASTM standard).
[c] 2% chloroform, acetone-D6 standard.

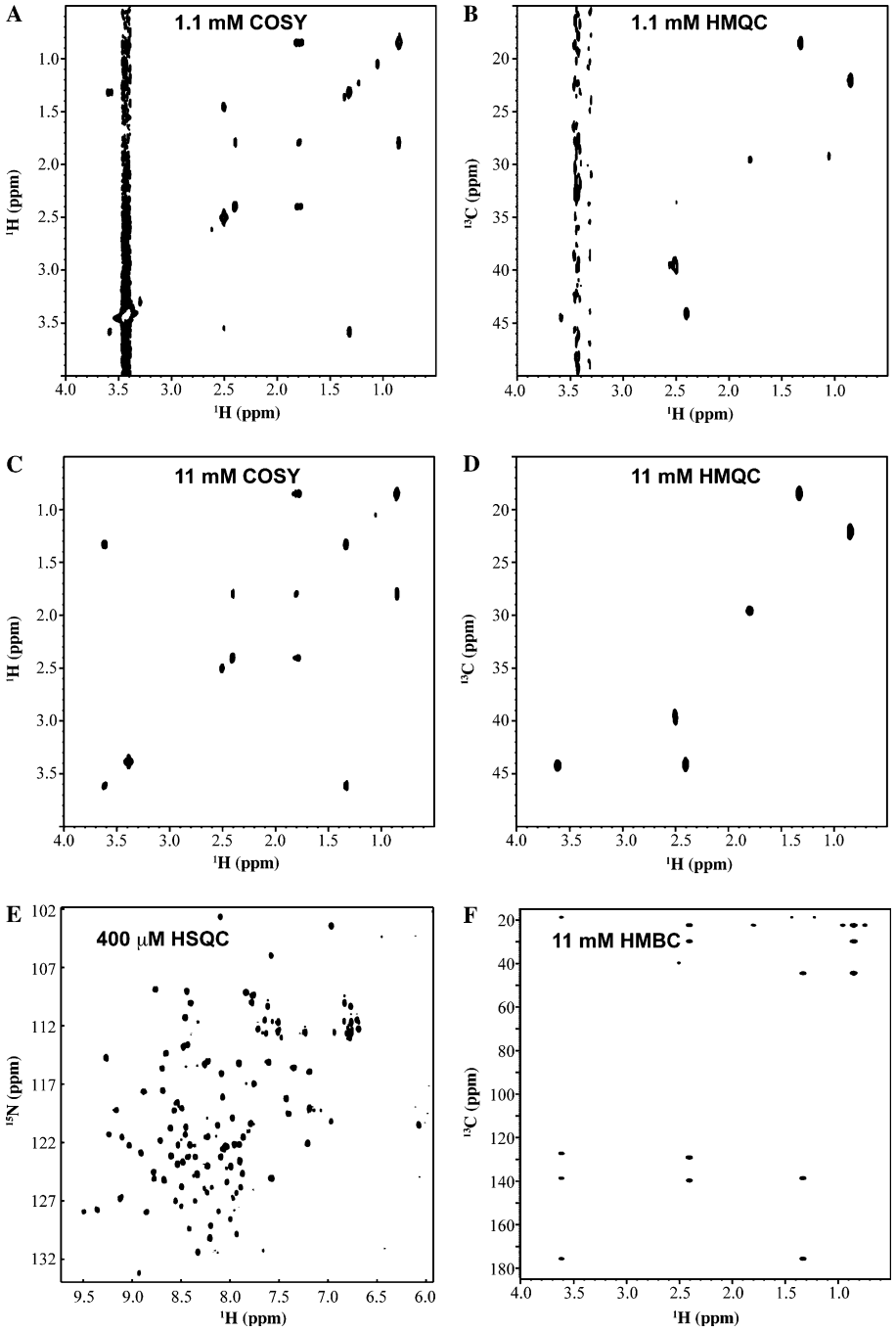

Fig. 2. Experimental data from the 1-mm HTS triple-resonance probe. (A and B) 1.7 μg ibuprofen (1.1 mM) in 7.5 μl DMSO-D6. High-resolution COSY (A) was recorded in 3 h 49 min using four scans and 1024 t_1 increments. ^{13}C-HMQC (B) was recorded with natural abundance ^{13}C in 16 h using 28 scans and 512 t_1 increments. (C, D, and F) 22.6 μg ibuprofen (11.1 mM) in 10 μl DMSO-D6. COSY (C) was recorded with the same parameters as (A). ^{13}C-HMQC (D) was recorded in 6 h 47 m using 12 scans and 512 t_1 increments. ^{13}C-HMBC (F) was recorded in 13 h 17 min using 32 scans and 464 t_1 increments. (E) 400 μM ^{15}N-ubiquitin in 8 μl phosphate buffer, 10% D_2O (pH 5.5). A gradient ^{15}N-HSQC (E) was recorded in 41 min using 16 scans and 128 t_1 increments.

parameters such as predicted versus experimental resonance frequencies.

The S/N value of 292 ± 28 for 0.1% ethylbenzene (Table 1) is approximately 3.5-fold less than a standard 600 MHz 5-mm triple-resonance probe ($S/N \sim 1000$) 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 values, approximately 4000 and 8000, respectively. The 1-mm HTS probe has a mass sensitivity that is over four times greater than a 5-mm cryogenic probe at the same field strength and over two times greater than state-of-the-art 5-mm technology at 800 MHz.

Microsolenoid probes have extremely high mass sensitivity because of optimal filling factors [2,3,8,9]. To compare the 1-mm HTS, we measured the anomeric ^{1}H from two different 10.0 mM preparations of sucrose in D_2O using 1, 4, and 8 fully relaxed scans. Each condition was repeated three times for a total of 18 measurements. These yielded a S/N per μmole per scan 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) [8]. Thus, the 1-mm HTS probe has slightly better or similar absolute mass sensitivity and much better total volume sensitivity than a microsolenoid probe at the same field strength.

The extremely high mass sensitivity of this probe suggests that it may be useful for metabolomics [10], natural products [11], and protein screening applications [12,13]. Fig. 2 shows experimental results for ibuprofen at natural ^{13}C isotopic abundance and ^{15}N-labeled ubiquitin. The ibuprofen spectra (Figs. 2A–D and F) were collected with either 22.6 μg in 10 μl DMSO-D6 (11 mM) or 1.7 μg in 7.5 μl DMSO-D6 (1.1 mM). The ubiquitin ^{15}N-HSQC spectrum (Fig. 2E) was collected with 400 μM ubiquitin in 8 μl phosphate buffer (pH 5.5) with 10% D_2O for lock.

The 11 mM ibuprofen spectra had extremely good S/N, including the natural abundance ^{13}C-HMBC. We were also able to easily collect standard ^{1}H 1D, high-resolution COSY, and ^{13}C-HMQC data on 1.1 mM ibuprofen. High-quality ^{15}N-HSQC spectra from 1 mM (data not shown) and 400 μM ubiquitin were collected in 10 and 41 min, respectively (Fig. 2E).

These results demonstrate that the 1-mm HTS triple-resonance probe is capable of very high-sensitivity measurements of small molecules and proteins. Although the absolute sensitivity makes it an impractical choice for full protein structural analysis, it can provide an economical screening platform for protein expression [12] and SAR-by-NMR [13] by requiring as little as 3 nmol of total protein at a concentration of less than 500 μM for each experiment.

Acknowledgments

Funding was from the NIH/NCRR (5P41RR016105-05). Werner Maas and Mark Chaykovsky at Bruker Biospin provided support and encouragement. Simulations were performed at the NHMFL and supported by the NSF (DMR 00884173) and State of Florida. NMR experiments were done in the AMRIS facility of the McKnight Brain Institute. We thank Dr. Cherian Zachariah for sample preparation, Dan Plant for NMR assistance, and Prof. Andrew Webb for helpful comments. The authors have no financial conflict of interest to report. W.B. and S.S. designed ^{1}H and ^{15}N coils and did simulations. R.W. and R.N. constructed the probe, and W.B. assisted with tuning coils. J.R. and A.E. did experimental measurements. A.E. obtained funding, prepared the manuscript, and coordinated the project.

References

[1] D. Hoult, R. Richards, Signal-to-noise ratio of nuclear magnetic resonance experiment, J. Magn. Reson. 24 (1976) 71–85.
[2] D.L. Olson, T.L. Peck, A.G. Webb, R.L. Magin, J.V. Sweedler, High-resolution microcoil H-l-NMR for mass-limited, nanoliter-vol Samples, Science 270 (1995) 1967–1970.
[3] Y. Li, T.M. Logan, A.S. Edison, A.G. Webb, Design of small volume HX and triple-resonance probes for improved limits of detection in protein NMR experiments, J. Magn. Reson. 164 (2003) 128–135.
[4] H. Kovacs, D. Moskau, M. Spraul, Cryogenically cooled probes - a leap in NMR technology, Prog. NMR Spectrosc. 46 (2005) 131–155.
[5] W.A. Anderson, W.W. Brey, A.L. Brooke, B. Cole, K.A. Delin, L.F. Fuks, H.D.W. Hill, M.E. Johanson, V. Kotsubo, R. Nast, R.S. Withers, W.H. Wong, High-sensitivity NMR spectroscopy probe using superconductive coils, Bull. Magn. Reson. 17 (1995) 98–102.
[6] H.D.W. Hill, Improved sensitivity of NMR spectroscopy probes by use of high-temperature superconductive detection coils, IEEE Trans. Appl. Supercon. 7 (1997) 3750–3755.
[7] W.W. Brey, W. Anderson, W.H. Wong, L.F. Fuks, V.Y. Kotsubo, R.S. Withers, US patent 5,565,778, 1996.
[8] D.L. Olson, J.A. Norcross, M. O'Neil-Johnson, P.F. Molitor, D.J. Detlefsen, A.G. Wilson, T.L. Peck, Microflow NMR: concepts and capabilities, Anal. Chem. 76 (2004) 2966–2974.
[9] W. Peti, J. Norcross, G. Eldridge, M. O'Neil-Johnson, Biomolecular NMR using a microcoil NMR probe—new technique for the chemical shift assignment of aromatic side chains in proteins, J. Am. Chem. Soc. 126 (2004) 5873–5878.
[10] O. Corcoran, M. Spraul, LC-NMR-MS in drug discovery, DDT 8 (2003) 624–631.
[11] M. Gronquist, J. Meinwald, T. Eisner, F.C. Schroeder, Exploring uncharted terrain in Nature's structure space using capillary NMR spectroscopy: 13 steroids from 50 fireflies, J. Am. Chem. Soc. 127 (2005) 10810–10811.
[12] S. Yokoyama, Protein expression systems for structural genomics and proteomics, Curr. Opin. Chem. Biol. 7 (2003) 39–43.
[13] S.B. Shuker, P.J. Hajduk, R.P. Meadows, S.W. Fesik, Discovering high-affinity ligands for proteins: SAR by NMR, Science 274 (1996) 1531–1534.