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Probes for high field solid-state NMR of lossy biological samples

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ABSTRACT

In solid-state NMR hydrated samples of biopolymers are susceptible to radio frequency heating and have a significant impact on probe tuning frequency and performance parameters such as sensitivity. These considerations are increasingly important as magnetic field strengths increase with improved magnet technology. Recent developments in the design, construction, and performance of probes for solid-state NMR experiments on stationary lossy biological samples at high magnetic fields are reviewed.

1. Introduction

Most proteins express their biological functions as constituents of supramolecular assemblies immersed in a salt-containing aqueous environment. Since the individual protein subunits are immobilized within the assemblies, both high magnetic fields and high power radio frequency (RF) irradiations are needed to obtain high-resolution spectra on stationary, aligned samples. However, these electrically lossy samples are problematic for the performance of NMR probes tuned for the high resonance frequencies. As a result, it has been necessary to rethink the design and construction of the multiple-resonance probes used for solid-state NMR studies of proteins.

In NMR spectroscopy, the term “solid-state” does not describe the physical state of the sample. Rather, it refers to the instrumentation and experimental methods that deal with un-averaged, anisotropic (static) nuclear spin interactions present in molecules that are immobile on timescales that are long compared to the inverse of the frequency spans of these interactions. In a typical sample of a membrane protein suitable for solid-state NMR experiments, the polypeptides are associated with liquid crystalline phospholipid bilayers in excess water, and such samples will have the appearance of a clear solution at room temperature. However, solution NMR spectra of these samples contain no or very weak, broad resonances because of the lack of sufficient motional averaging. Only the high power radio frequency irradiations of multiple-pulse and double-resonance solid-state NMR experiments are capable of averaging the spin interactions and yielding high-resolution NMR spectra.

There are two basic classes of designs for probes capable of performing solid-state NMR experiments. Either a single sample resonator, such as a solenoid coil, tuned to multiple frequencies, or multiple sample resonators nested in a cross-coil [1] configuration, each of which is tuned to one or two frequencies, are used. The trade-offs between these two designs are complex and the better choice depends upon the specific requirements of the samples and experiments. At high fields, there are large frequency differences between the ^{1}H and the low gamma (^{13}C and ^{15}N) nuclei, which makes the compromises inherent in double- or triple-tuned single coils more costly, and the use of multiple resonators more attractive, since individual resonators can be optimized for their frequency of operation, and complexity of the tuning circuits is reduced by relying in-part on physical separation rather than solely on circuit elements, such as traps and filters, to provide the requisite isolation of the frequencies. An extreme example of the multiple resonator probe concept is represented by Electron Paramagnetic Resonance (EPR) probes designed to perform Electron Nuclear Double Resonance (ENDOR) experiments where the only feasible choice is to use individual resonators optimized for the gigahertz electron resonance and the megahertz nuclear resonances frequencies [2]. The drawbacks of using multiple resonator probes are chiefly geometric, because of the necessity of generating orthogonal fields with cross-coils and the unavoidable reduction of the filling factor for the outer resonator in a nested configuration. In solid-state NMR, traditional probe designs relied on a single solenoid coil tuned to multiple frequencies, which works well for relatively low frequencies and crystalline samples. However, as the frequency disparity increases in high field magnets, the trade-offs associated with any given value of solenoid coil inductance become more pronounced, especially for lossy aqueous samples of biopolymers.

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The interactions between an electrically lossy sample and the electrical fields generated in the tuned resonator by high frequency RF irradiations result in sample heating and dramatic changes in the performance of the probe, especially a loss of sensitivity [3,4]. Two general strategies have been pursued to mitigate the deleterious effects of the lossy sample upon probe performance, both of which reduce the undesirable electrical effects and the sample heating that originate mostly in the conservative electric fields generated by a solenoid coil. The conservative electric field generated within the sample volume is proportional to the coil inductance, or with all other parameters held constant, the number of turns of the solenoid coil. A scroll coil provides a reduced electric field at the sample by virtue of its design, which features a built-in capacitance as well as a relatively low inductance [5,6]. We have described a ^{1}H/^{31}P double-resonance scroll coil probe that takes advantage of the favorable high frequency performance of this design. Other types of resonators have been introduced that reduce RF heating by minimizing the electric fields within the sample volume [7,8]. We recently described an approach that enables solenoid coils to be used with lossy samples; based on the principles of a Faraday shield [9], a strip-shield insert [10] placed between the sample and the coil localizes the undesirable conservative electric fields outside the sample volume, effectively shielding the sample from the heating effects of the RF irradiations [11,12]. An alternative approach uses a low inductance resonator tuned to the high ^{1}H frequency while employing a solenoid coil for the low frequency channels in a cross-coil configuration [13–17]. We have described a cross-coil double-resonance probe that uses an outer Modified Alderman-Grant Coil (MAGC) tuned to the ^{1}H frequency and an inner solenoid coil tuned to the low gamma ^{15}N frequency [18]. Other groups have recently described a number of other promising approaches to probes for solid-state NMR of lossy samples at high fields [8,19,20]. In this review we summarize recent progress in probe development and compare the various designs.

2. Probe

As the interface between the sample and the weak magnetic fields generated by the RF irradiations, the probe is the spectrometer component with the strongest influence on the overall performance of the experiments. Besides providing a mechanical platform for positioning or rotating the sample and controlling its temperature, the probe consists of one or more resonators located concentrically around the sample that are tuned by capacitors and other circuit elements to the resonance frequencies of the nuclei of interest. Superconducting magnets are now capable of generating homogeneous, stable magnetic fields at strengths (23T) that correspond to ^{1}H resonance frequencies (1 GHz) in the middle of the Ultra High Frequency (UHF) (300 MHz–3 GHz) band, and higher fields are in the offing as magnet technology continues to develop. At these field strengths, solid-state NMR experiments on stationary samples are extremely demanding because of the high RF fields needed to perform homo- and hetero-nuclear decoupling across broad bandwidths for the entire duration of an exper-

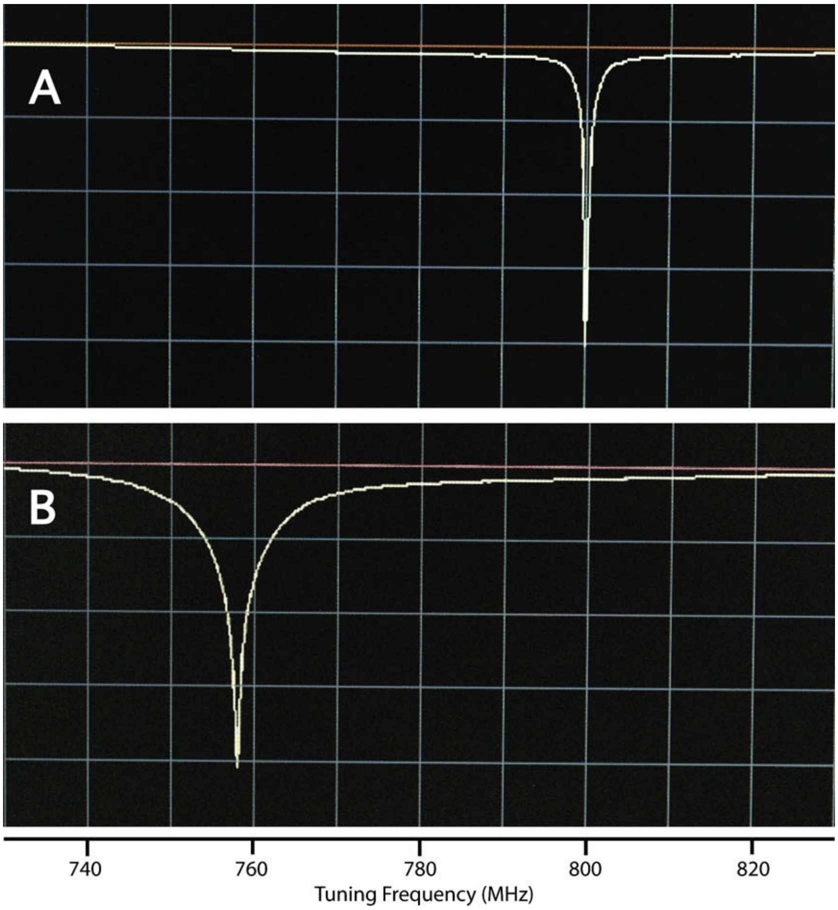

Fig. 1. Network analyzer screen shots for a circuit with a solenoid sample coil tuned to 800 MHz (A). The empty solenoid coil has a resonance center frequency of 800 MHz and Q of 160 (B). The same solenoid coil containing a typical lossy sample (160 μl of 70 mM NaCl solution) which shifts the resonance center frequency to 759 MHz and lowers the Q to 27.

iment without the assistance of averaging due to mechanical rotation of the sample at the magic angle.

Probes must meet the conflicting demands presented by electrically lossy samples and irradiations with intense radio frequency fields at high frequencies. Two aspects of the problem are illustrated in Fig. 1. The insertion of a lossy sample that mimics the properties of protein-containing phospholipid bilayers into an empty solenoid coil tuned to 800 MHz induces a significant (41 MHz) downward shift of the tuning frequency. By itself, this shift presents a challenge in circuit design. The relative breadth of the signals in Fig. 1 shows that the introduction of a lossy sample lowers the quality (Q) factor of the circuit from 160 to 27, which results in a dramatic loss of probe efficiency and sensitivity. Moreover, the frequency shift and Q reduction are accompanied by substantial sample heating from the RF irradiations which interact strongly with the conductive sample [21], particularly at the high ^{1}H resonance frequency but also at the lower ^{13}C frequency. Taken together, these factors preclude the use of conventional solenoid coils at ^{1}H resonance frequencies.

In early contributions [4], Gadian and others attributed these effects to dielectric loss as well as the conductivity of the sample. They showed that the interaction of the scalar (conservative) electric field was the dominant mechanism of the deleterious interactions between the coil and the conductive sample. Notably, Alderman and Grant [22] recognized that the scalar portion of the electric field is proportional to the voltage drop across the leads of the coil, and thus proportional to the inductance of the coil, which led to the substitution of low inductance resonators for solenoid coils in NMR probes.

The construction of probes capable of withstanding prolonged high power RF irradiations was an integral part of the development of the field of high-resolution solid-state NMR spectroscopy. The probes used in the initial double-resonance experiments had two single-tuned resonators in a cross-coil configuration, typically an outer Helmholtz coil tuned to the ^{1}H frequency and an inner solenoid coil tuned to the ^{13}C frequency as a mean of simplifying the overall circuit design [1]. For the next 25–30 years, most probes utilized a single, double- or triple-tuned solenoid coil in recognition of the elegance of such an approach, and the availability of circuits that offered simple and effective ways to electrically isolate the tuning frequencies. In the past few years, the necessity of dealing with lossy samples at high frequencies has resulted in a resurgence of interest in cross-coil probes, since it is possible to reduce the amount of sample heating from the ^{1}H resonator in this configuration by using a low inductance coil with a concomitantly low conservative electric field heating effect. The main disadvantage of cross-coil designs results from the low filling factor for the outer coil, which limits its ability to generate strong B_1 fields and reduces its sensitivity, although the reduced filling factor does contribute indirectly to a reduction of sample heating. Our conceptualization of coil selection is illustrated in Fig. 2, which plots probe performance on a lossy sample as a function of sample coil inductance, or if all other parameters remain constant, the number of turns in the coil. At high frequencies, the performance of the coil improves significantly for lossy samples as the inductance is decreased. Similarly, the sensitivity of the low frequency coil improves with increasing inductance within certain limits; wire resistance generally diminishes the advantage of 5 mm solenoid coils with more than 8 or 9 turns. For double or triple-tuned solenoid coils, Fig. 2 suggests that it is feasible to select a coil with an inductance that is a compromise between low frequency sensitivity and high frequency performance. For example, we have found that a 5 mm ID, five turn solenoid coil can be double-tuned to ^{1}H at 700 MHz and ^{15}N at 70 MHz, and give reasonable performance, albeit with sample heating and somewhat reduced ^{15}N sensitivity, in double-resonance experiments on lossy samples.

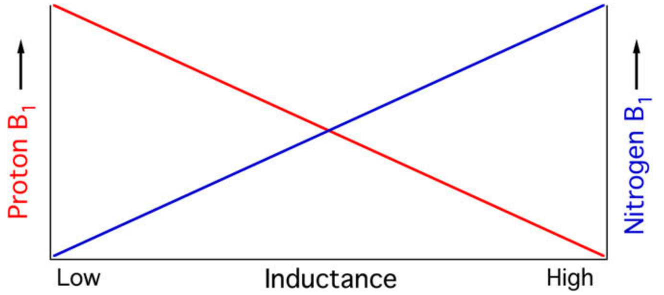

Fig. 2. A schematic illustration of the general trend of probe performance for a high frequency nucleus ^{1}H (red) and a low frequency nucleus ^{15}N (blue) as a function of coil inductance for lossy samples at high fields. Here low inductance represents the inductance of a one turn solenoid and high inductance represents the inductance of a nine turn solenoid. In general, proton performance improves as the coil inductance is reduced, while low frequency performance tends to improve as the inductance increases. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

RF heating is a well-characterized phenomenon that plagues all NMR experiments on lossy aqueous samples. Efforts to minimize sample heating have focused primarily on the resonator tuned to the ^{1}H frequency. However, significant heating can also result from irradiations at ^{13}C resonance frequencies at high fields, and this will become an increasingly important factor in the design and construction of probes for ^{1}H/^{13}C/^{15}N triple-resonance experiments in the future.

In this review, in order to make these considerations more concrete, we describe three probe designs that we have constructed and tested in the past few years as part of our solid-state NMR studies of proteins in supramolecular assemblies, such as virus particles and membranes. They should be equally applicable to other types of proteins that are immobile and hydrated. These designs include probes that incorporate a strip-shield solenoid coil, a scroll coil, and a Modified Alderman-Grant coil. In the summary we make comparisons to alternative probe designs for biological solid-state NMR experiments.

3. Solenoid coil

In the past, most solid-state NMR probes utilized a solenoid coil that was double- or triple- tuned using a Cross-Waugh type of circuit [23–27] or a variation that employed transmission lines as tuning elements [28,29]. The contemporary version of a Cross-Waugh circuit shown in Fig. 3A can be used to double tune a solenoid sample coil of inductance Ls. The circuit utilizes capacitors C1 and C4 for scaling; variable capacitor C2 tunes the ^{1}H circuit and variable capacitor C3 matches the ^{1}H circuit. Capacitor C5 and transmission line L1 together constitute the "quarter wave" element, which resonates at the ^{1}H frequency, and provides a ground for the low frequency channel, effectively isolating it from the ^{1}H tuning elements. An optional band stop filter utilizing capacitor C7 and inductor L2 may be used to isolate the ^{1}H channel from the low frequency X channel. Capacitor C6 serves as a voltage divider, variable capacitor C8 tunes the low frequency circuit, and fixed capacitor C9 shifts the low frequency resonance to the desired frequency. Variable capacitor C10 is the low frequency match capacitor, and inductor L3 and capacitor C11 form an LC trap resonating at the ^{1}H frequency to provide isolation at the ^{1}H frequency. Historically, the inductance of solenoid sample coils (Ls) with between 4 and 7 turns provided a reasonable compromise as described in Fig. 2 – high enough for the low frequency nuclei (^{13}C and ^{15}N) and low enough to accommodate tuning to the high frequency nucleus (^{1}H). A solenoid coil probe and a typical aqueous sample of a membrane protein in phospholipid bilayers are shown in Fig. 3. This design worked well in many chemical and biochemical appli-

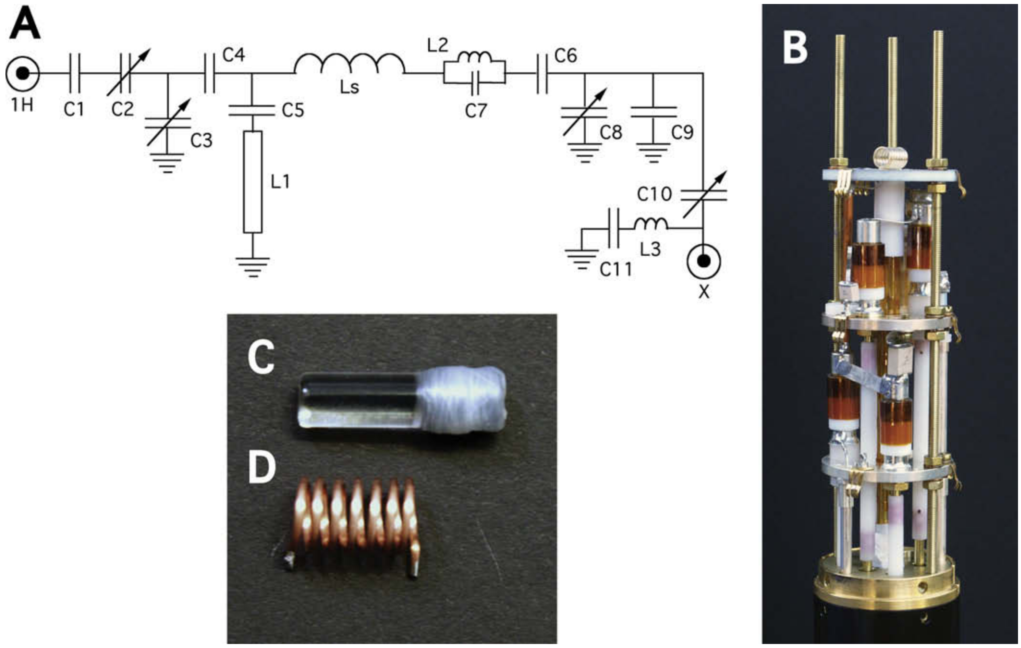

Fig. 3. (A) A typical modified Cross-Waugh double resonance solid-state NMR probe circuit where *X* represents the ^{15}N or ^{13}C channel. (B) A completed double-resonance solid-state NMR probe with a solenoid sample coil. The probe is shown without the probe cap, which encloses the circuit assembly. (C) A typical 5 mm OD membrane protein sample in hydrated phospholipid bicelles. (D) A multi-turn 5 mm ID, 1 cm long solenoid coil.

cations, and only became problematic when the combination of high magnetic fields and lossy biological samples resulted in the substantial frequency shifts and *Q* reduction illustrated in Fig. 1 and the severe sample heating that denatured samples.

It is possible to work around the sample-induced frequency shifts shown in Fig. 1 by extending the tuning range of the circuit, although in many cases this necessitates the replacement of fixed capacitors when samples are changed to allow for tuning to samples with a wide range of conductivities. However, desoldering and soldering delicate capacitors every time the sample is changed is not a practical approach. The problem of *Q* reduction also illustrated in Fig. 1 can be partially overcome by using higher RF power and longer signal averaging. However, the sample heating can be intractable, even with the use of long recycle delays that exacerbate the sensitivity problems. Although all three effects are linked, sample heating has received the most attention because of its devastating effects on the samples. The most direct way to reduce the RF heating is to reduce the inductance of the coil and, therefore, the scalar electrical field generated within the sample. While a low inductance coil is desirable for the ^{1}H resonance frequency, at high field strengths, the large gap between the frequencies of ^{1}H and ^{13}C and especially ^{15}N place competing demands on the choice of inductance of solenoid coils in double or triple tuned configurations. At moderate ^{1}H resonance frequencies (<700 MHz), it is possible to work with a coil with a somewhat reduced coil inductance that results in acceptable performance for ^{13}C and ^{15}N and tolerable sample heating that can be managed with long recycle delays. This enables the flexibility of solenoid coils to be available for specific experiments. For example, we have constructed double-resonance probes with relatively large “flat” solenoid coils [30] in order to optimize the filling factor for mechanically-oriented samples of membrane proteins on glass plates as well as a platform for tilted coil experiments [31].

4. Strip-shield

Although a double- or triple-tuned solenoid coil can perform well with non-lossy samples, such as anhydrous crystalline materials in high magnetic fields, the effects of lossy aqueous samples on tuning, quality factor, and sample heating are limiting. A strip-shield [10] combats these effects while preserving the favorable characteristics of the solenoid coil. As shown in Figs. 4 and 5, a strip-shield is a thin tube inserted between the sample and the solenoid coil; it locates thin copper strips along the long axis of the coil, parallel to the B_1 field generated by the RF irradiation. The copper strips are encapsulated in a dielectric material (polytetrafluoroethylene (PTFE)) to prevent arcing. The shield, in effect, sequesters the undesirable electric fields into the region of the conductive copper strips, and away from the sample.

Fig. 4 illustrates the principal features of a strip-shield. Since the strips and the dielectric material reduce the filling factor of the coil by requiring a somewhat larger inner diameter (5.6 mm) than required by the outer diameter of the sample (5 mm), it is desirable to utilize the thinnest shield that is practical. Following standard practice, the single solenoid coil is double- or triple- tuned to the resonance frequencies of interest. A complete double-resonance probe based on a modified version of the Cross-Waugh circuit is shown in Fig. 5. Unlike many cross-coil designs [13,14] where the electric field reduction only affects the ^{1}H channel, in this design all channels benefit. At the higher ^{1}H and ^{13}C resonance frequencies, the improvement in *Q* for a lossy sample more than compensates for the loss in filling factor in terms of the magnitudes of the B_1 fields generated at a specified power level, and sensitivity, and the strip-shield reduces RF heating by a factor of 5–7. At the ^{15}N frequency, the performance of the strip-shield containing coil is similar to that of a stand-alone solenoid coil.

5. Scroll coil

With lossy samples a scroll coil offers several advantages over a solenoid coil of similar size. Foremost are the increased efficiency and sensitivity, and reduced sample heating at the high ^{1}H resonance frequency. In addition, the B_1 homogeneity is very high without the need for a sophisticated radio frequency circuit, making circuit design straightforward. It is an excellent choice for ^{1}H-detected experiments where high frequency ^{1}H performance is of

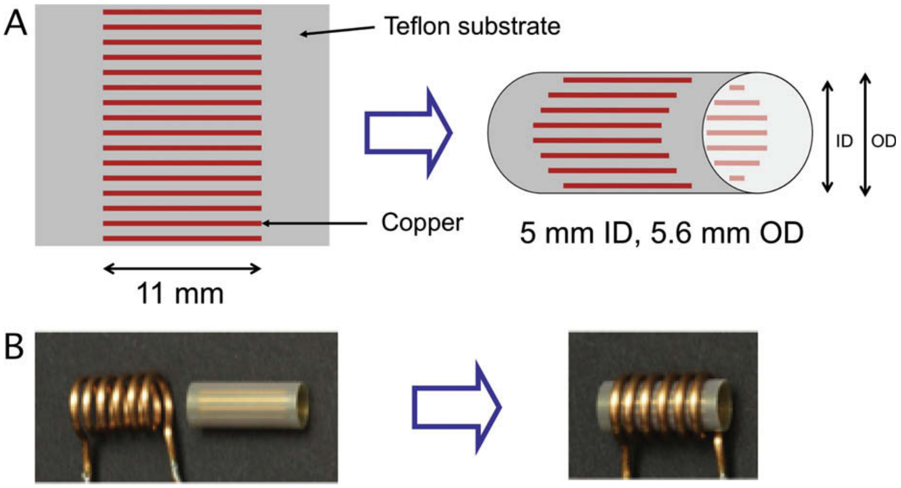

Fig. 4. (A) An illustration of the strip-shield coil liner consisting of copper strips supported on a PTFE substrate, which is rolled up to form a coil liner that accommodates a 5 mm OD sample tube and fits within a 5.6 mm ID solenoid coil. (B) The strip-shield is inserted into multi-turn solenoid coil as a liner between the sample, (as shown in Fig. 3C) and the solenoid coil.

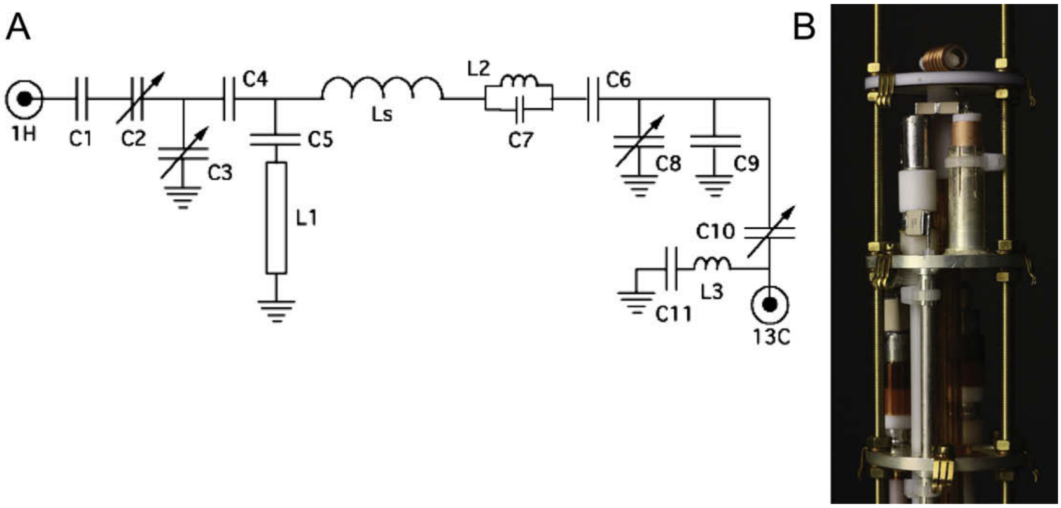

Fig. 5. (A) The modified Cross-Waugh circuit [24] used to double tune the solenoid coil of a strip-shield probe to ^{13}C and ^{1}H. Ls represents the sample coil/strip-shield assembly. (B) Assembled probe shown with a sample loaded into the sample coil.

paramount importance. The most significant drawback to the scroll coil is the markedly reduced efficiency and sensitivity at the lower ^{13}C and ^{15}N resonance frequencies.

A scroll coil consists of a single sheet of copper wrapped concentrically with a non-conducting dielectric material, such as PTFE, as shown in Fig. 6. Because of the small electric field generated within the sample volume of a scroll coil, sample heating is minimal, and probe tuning is not significantly perturbed by lossy samples. As a result, the probe has sufficient flexibility to be used to test pulse sequences on single crystal or powder samples, and then to be used to study aqueous protein samples without replacing fixed capacitors or other tuning elements.

A scroll coil has lower sensitivity than a solenoid coil for directly-detected ^{13}C or ^{15}N experiments because of its marginal low frequency performance; however, we have found that for higher frequency operations in a ^{1}H/^{31}P double-resonance probe, the scroll coil is truly outstanding. Most applications of the scroll coil in NMR probes have been for ^{1}H/^{13}C/^{15}N triple-resonance magic angle sample spinning experiments, where it has been suggested that the high B_1 homogeneity of the scroll coil leads to more effective cross-polarization which may somewhat offset the low sensitivity for directly-detected ^{15}N and ^{13}C experiments [5,6].

6. Modified Alderman-Grant Coil (MAGC)

The compromises inherent in double- or triple- tuned solenoid or scroll coils, can be largely avoided in cross-coil probes with two separate resonators and tuning circuits. With this approach, one resonator is optimized for the high frequency of ^{1}H and the other for the lower frequencies of ^{15}N and/or ^{13}C. This results in a probe that generates little RF heating because the outer resonator tuned to the high ^{1}H frequency has low inductance and a small filling factor. High efficiency and sensitivity for the ^{15}N and ^{13}C frequencies result from the high inductance and filling factor of the inner solenoid coil holding the sample, although the ^{13}C channel retains the sample heating properties of a solenoid coil.

In the early versions of cross-coil probes, the outer ^{1}H resonator was a Helmholtz coil [1], however, this resonator is obsolete because of its poor performance at high frequencies and marginal RF homogeneity. A Modified Alderman-Grant coil has much better performance characteristics. Its low inductance ensures a minimal voltage drop across the resonator, and thus the sample. The MAGC shown in Fig. 7 was machined from a solid rod of oxygen free copper, which enabled its geometry to be optimized to create a homogenous B_1 field in the central region where a 5 mm ID sole-

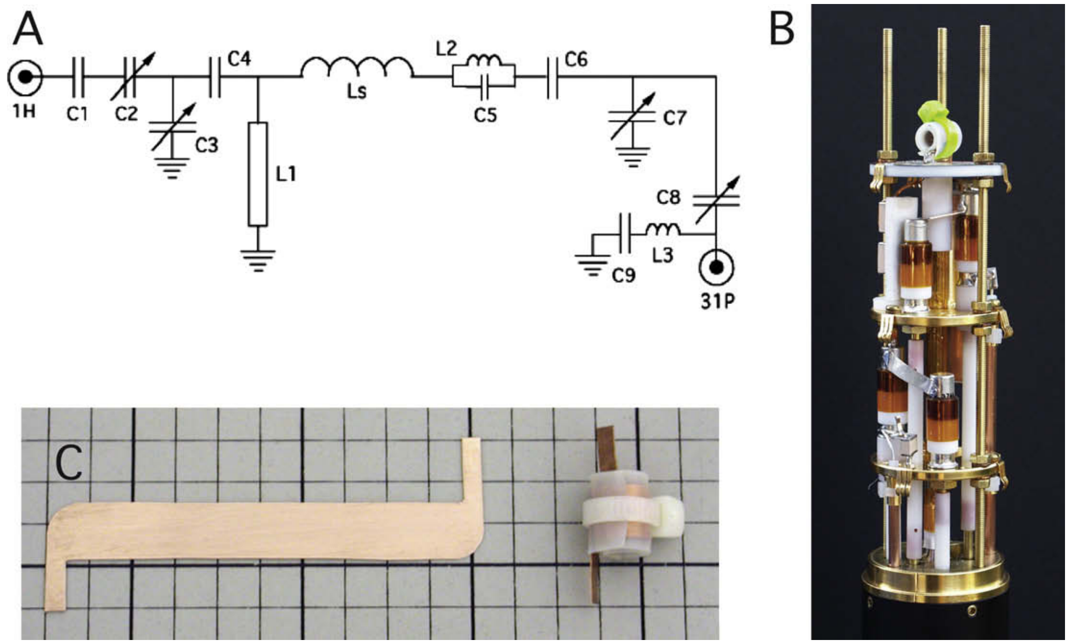

Fig. 6. (A) Tuning circuit for a 700 MHz ^{1}H–^{31}P scroll coil probe where Ls represents the scroll coil. (B) The complete assembly without probe cap. (C) Copper blank measuring 2.36 in. between the inner edges of the leads used to fabricate a scroll coil. A plastic cable tie cinch on the outside is used to stabilize the completed scroll coil assembly.

noid coil is located in the assembled probe. With the capacitors in place, the resonator produces a B_1 field orthogonal to its long axis, through the window [32,33], and the inner solenoid coil produces its B_1 field along the long axis of the solenoid/MAGC pair. The simplified tuning circuit and inductance optimized for its frequencies of operation ensure excellent sensitivity in ^{13}C and ^{15}N direct-detection experiments. It is essential to balance [34] the circuit in order for the MAGC to exhibit optimal efficiency, homogeneity,

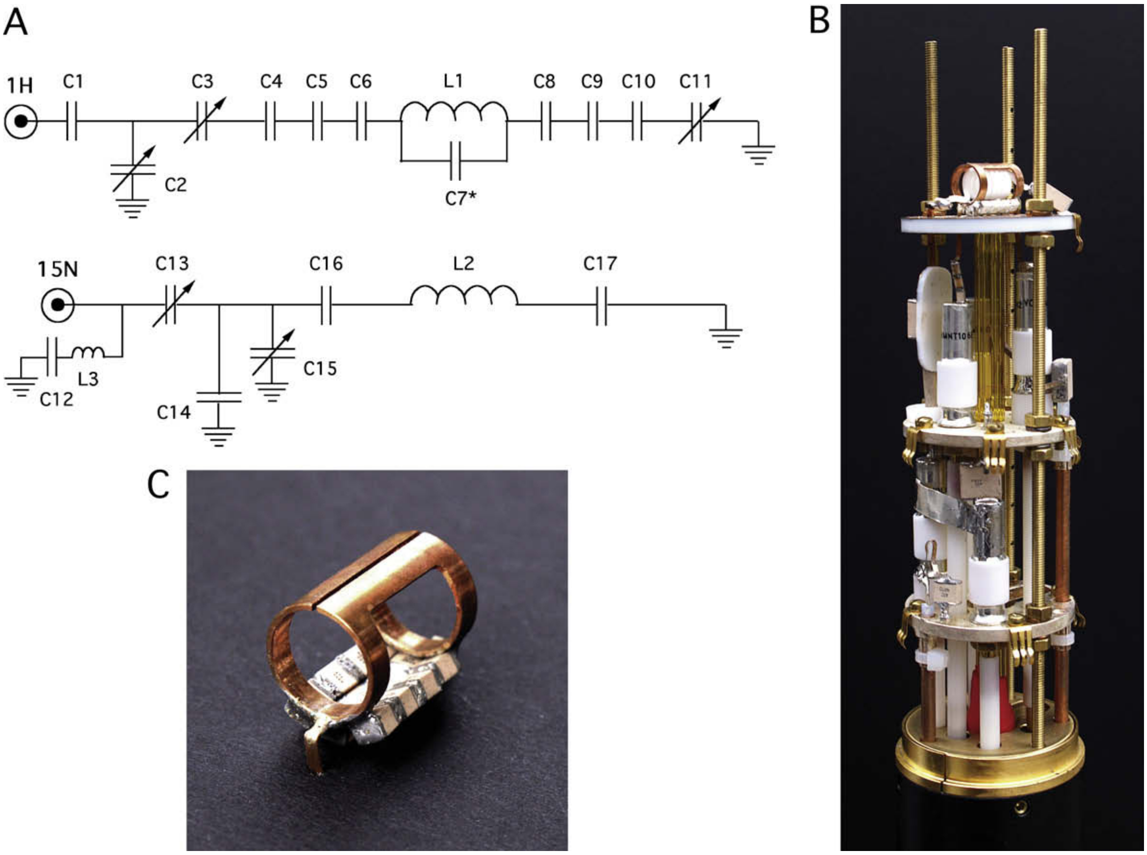

Fig. 7. (A) Cross-coil probe utilizing two individual coils tuned using the circuit displayed. The ^{1}H coil (L1) is the outer most coil and consists of a Modified Alderman-Grant coil (bottom) and an inner solenoid coil is represented as L2. (B) The completed assembly without probe cap. (C) A Modified Alderman-Grant coil and the capacitors that constitute C7* in the circuit.

and power handling capabilities, but this is a straightforward process for a single resonance circuit.

Overall, using a MAGC as the outer coil in a cross-coil probe offers several advantages. The low inductance of the MAGC coil is very effective at reducing RF heating due to irradiation at the high ^{1}H frequency (Fig. 8). The simple tuning circuit utilizes a minimum number of tuning elements for the inner coil and provides the flexibility to choose an inner coil of optimal inductance for the low frequency detection channel. This contributes to good sensitivity in direct-detection experiments. The outer MAGC is relatively compact, which improves the performance of the ^{1}H channel, and results in a compact overall resonator structure that fits inside narrow bore magnets. The main disadvantage of this design (and present in all cross-coil designs) results from the very same properties of the MAGC resonator that minimize the RF heating, namely the low inductance of the MAGC and its relatively low filling factor, which render the coil insensitive for direct observation of ^{1}H signals. However, the design could be reversed, and cross-coil designs with an inner ^{1}H resonator and outer low frequency resonator have been described [13,17], which would sacrifice low frequency sensitivity for increased ^{1}H performance while still retaining the favorable RF heating properties of a low inductance ^{1}H resonator. Taken together, the advantages resulting from the compact cross-coil design and the optimization of the respective high and low frequency coils make this a good choice for many studies of lossy biology samples by solid-state NMR.

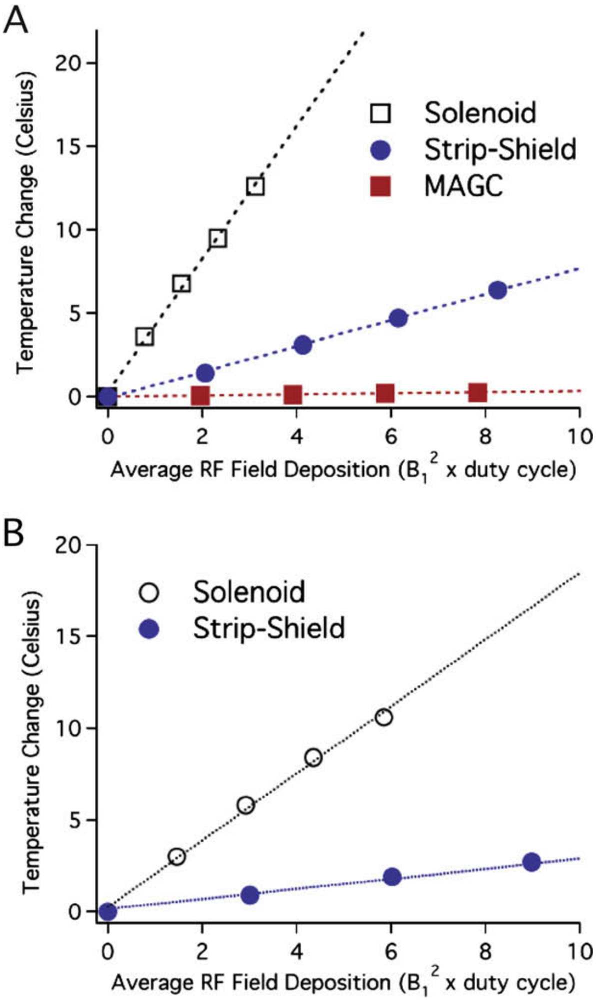

Fig. 8. (A) ^{1}H RF heating data that compares a solenoid coil probe with a strip-shield probe and an MAGC probe. The strip-shield results in a 5-fold reduction and the MAGC coil results in a 20-fold reduction in RF heating. (B) ^{13}C RF heating data for the strip-shield probe demonstrates a 7-fold reduction in RF heating.

7. Comparison of resonators

Reducing the RF heating of samples is important not only because of the potential for denaturing the proteins, but also because RF heating is not uniform across the sample volume, and even modest temperature gradients can broaden resonances and effectively reduce both resolution and sensitivity [35]. RF heating for the strip-shield and MAGC probe designs are compared in Fig. 8. Sample temperature is plotted as a function of the average RF field deposition, calculated as the product of the square of B_1 and the duty cycle factor. The duty cycle is the ratio of the time that the RF irradiation is on to the total duration of the pulse sequence, and average RF field deposition values for ^{1}H range from 4–10 for typical double-resonance multidimensional solid-state NMR experiments, and up to about 2.5 for the low frequency channels. For the ^{1}H channel, RF heating is reduced 5-fold and 20-fold for the strip-shield and MAGC, respectively. The MAGC results in minimal sample heating due to the shielding effects provided by the inner coil in addition to the relatively small filling factor for the outer resonator. The strip-shield also offers a 7-fold reduction in ^{13}C RF heating over a conventional solenoid coil, such as that employed in the MAGC, which may be significant in triple-resonance experiments that incorporate ^{13}C decoupling during signal acquisition. The scroll coil was shown to have favorable heating characteristics for a very wide range of sample ionic strengths [5]. In our implementation on a ^{1}H/^{31}P probe, we found that it provided a 6.5-fold reduction in ^{1}H RF heating [6].

Optimal resolution and sensitivity in solid-state NMR experiments on proteins are obtained at high magnetic fields. A conventional solenoid coil has excellent performance characteristics and can be tuned to multiple frequencies. However, the insertion of a lossy aqueous sample impairs its performance in several ways. These effects are strongest at frequencies >700 MHz, but can also impact the performance at lower frequencies. The most noticeable effects are a large change in the center frequency and reduction of the *Q* of the circuit (Fig. 1). While tuning the probe for lossy samples rather than an empty coil can compensate for this, it limits the ability to switch between lossy and non-lossy samples to tune and set up the spectrometer system for complex experiments. Increasing the tuning range to accommodate both lossy and non-lossy samples often results in unstable tuning. Equally deleterious effects result from the lowering of the *Q* of the circuit, which decreases the efficiency of the RF irradiations and the sensitivity of the probe. A complicating factor is that the individual high (^{1}H) and low (^{15}N, ^{13}C) frequency channels in double- or triple- tuned probes are differentially affected by lossy samples at high fields, with a stronger effect on the higher frequency channels. The severe heating of lossy samples by high frequency RF irradiation can destroy samples. This has been addressed by several recent developments, principally through the use of alternative coil geometries in the probes [8,13,35].

The strengths and weaknesses of the various coils used in probes for solid-state NMR of proteins are compared in Fig. 9. In general, for samples that are not lossy, the solenoid coil offers the best overall performance but would be the worst choice for analysis of lossy samples. Cross-coil probes have sensitivity advantages, since the inductance and filling factor of the inner resonator are optimized for the lower frequency resonances that are detected, and because of the simplified single resonance circuits that are employed to the individual coils. The scroll coil would likely be the best choice for ^{1}H detected experiments in situations where the reduced B_1 capabilities and sensitivity of the lower frequency

	A	B	C	D	E	F
B_1 Efficiency ^{1}H	★★☆☆☆	★★☆☆☆	★★☆☆☆	★★★★★	★★★★☆	★★★★☆
B_1 Efficiency ^{15}N ,^{13}C	★★★★☆	★★★★★	★★★★★	★★☆☆☆	★★☆☆☆	★★★★☆
Homogeneity ^{1}H	★★★☆☆	★★★★☆	★★★★☆	★★★★★	★★★★☆	★★★★☆
Homogeneity ^{15}N ,^{13}C	★★★☆☆	★★★★☆	★★★★☆	★★★★☆	★★★★☆	★★★★☆
RF heating ^{1}H	★☆☆☆☆	★★★★★	★★★★★	★★★★★	★★★★☆	★★★★☆
RF heating ^{15}N ,^{13}C	★☆☆☆☆	★☆☆☆☆	★☆☆☆☆	★★★★★	★★★★☆	★★★★☆

Fig. 9. Comparison of performance attributes of various coil designs when loaded with lossy samples. (A) solenoid coil [23,36], (B) low-E resonator [14], (C) MAGC [18], (D) scroll coil [5], (E) loop-gap loaded coil [8], (F) strip-shield [10]. In general, cross-coil probes sacrifice performance of the outer most resonator. The scroll coil offers some of the most impressive ^{1}H performance, but low frequency performance is hampered by the overall low inductance. The strip-shield offers an excellent balance of performance attributes, but does sacrifice low frequency sensitivity.

channels can be tolerated. The strip-shield-containing solenoid coil offers excellent RF heating characteristics, and provides a significant improvement in overall ^{1}H and ^{13}C performance with lossy samples at a small cost of ^{15}N performance. Moreover, the strip-shield reduces RF heating at the ^{13}C frequency, which may well become an increasingly important factor as higher field magnets are developed.

The probe designs compared in Fig. 9 are all aimed at improving performance characteristics for the study of lossy samples at high magnetic fields, where RF heating and the effects of lossy samples on probe sensitivity can be overwhelmingly for conventional solenoid coils. For the most part, these are relatively recent developments built upon principals that have been discussed in the literature and informally among spectroscopists for decades. There is no single optimal probe configuration for solid-state NMR of lossy biological samples. However, the design, construction, and testing of probes is an active area of research and the pace of improvements is increasing to match the unrivalled potential of solid-state NMR to study proteins under physiological conditions.

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