

## A rotatable flat coil for static solid-state nuclear magnetic resonance spectroscopy

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A holder allowing rotation of a flat coil relative to the static magnetic field ( $\mathbf{B}_0$ ) of a nuclear magnetic resonance (NMR) spectrometer over the range of orientations from parallel to perpendicular has been built and incorporated into a two-channel wide line static solid-state NMR probe. Radiofrequency induction by the coil is perpendicular to  $\mathbf{B}_0$  for all rotational angles. Such a coil holder provides ready access to an additional directional variable for experiments exploiting sample orientation such as those using planar-supported oriented phospholipid bilayers. Rotation of the flat coil is demonstrated using  $^{31}\text{P}$  NMR of oriented phospholipid and peptide bilayers sandwiched between pairs of mica substrates. © 2005 American Institute of Physics.

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The effects of the orientation dependence of the chemical shift, dipole-dipole, and quadrupolar interactions in the solid state tend to broaden nuclear magnetic resonance (NMR) signals such that the resolution of individual resonances is impractical. High-speed sample spinning at the so-called magic angle is frequently used to improve resolution by averaging away tensorial interactions.<sup>1,2</sup> Inherent anisotropy is seen in cases such as single crystals, orientationally ordered polymers, liquid crystals, and planar-supported phospholipid bilayers. In this situation, static solid-state NMR experiments (i.e., those where the sample is static relative to the magnetic field of the spectrometer,  $\mathbf{B}_0$ ) provide a wealth of information concerning molecular orientations or properties of individual classes of nuclei within the sample.<sup>2-4</sup>

Oriented lipid bilayers, with or without incorporated membrane-spanning peptides or proteins, may be prepared on thin planar supports such as glass<sup>3,4</sup> or mica.<sup>5</sup> The charged head group of a phospholipid readily adsorbs to either of these hydrophilic substrates in the presence of water, promoting the formation of a bilayer on the substrate. The addition of lipids in excess of a single bilayer results in ordered multilayer formation, with hydrophilic head groups of each subsequent bilayer adsorbing to those of the previous bilayer. Stacked substrates are typically used to provide a reasonable signal-to-noise ratio for NMR spectroscopy. Such samples are square or rectangular in cross section and, even with dozens of substrates in the stack, tend to be relatively short in height.

Oriented solid supported samples may be placed in a solenoid radiofrequency coil used for static solid-state NMR. However, most of the cylinder cross section will then be unoccupied and the filling factor [the proportion of the radiofrequency (rf) coil volume occupied by a sample] will suffer, with a directly proportional decrease in signal

intensity.<sup>6</sup> Flat coils were introduced by Bechinger and Opella to combat this decrease in signal.<sup>7</sup> These coils have a rectangular cross section just large enough to fit the sample (Fig. 1). Decreases in the filling factor arise only from any material used to wrap the sample and from the volume occupied by the substrates themselves. Although typically used for oriented lipid bilayer samples, such a coil would also be appropriate for a study of aligned fibers embedded in resin, oriented polymer films, or liquid crystals.

A variety of information may be obtained by changing the orientation from that shown in Fig. 1, where the sample plane normal is perpendicular to  $\mathbf{B}_0$ .<sup>8</sup> In order to take advantage of the improved sensitivity of a flat coil while allowing the sample orientation to be changed relative to  $\mathbf{B}_0$ , several aspects of design need to be taken into account, in addition to the space limitations imposed by the magnet bore and probe body. First, the rotation of the coil must be such that the direction of the rf field generated or observed by the coil ( $\mathbf{B}_1$  in Fig. 1) remains perpendicular to  $\mathbf{B}_0$ . Second, at least one end of the coil must be fully accessible for sample insertion, removal, or adjustment. Third, a variation of the coil rotation angle should be possible without disconnection of the coil holder from the probe. Fourth, the coil itself should be exchangeable. Fifth, the holder needs to remain sturdy and fixed in position upon the attachment of variable-temperature unit interfaces and the insertion into the magnet bore. Finally, the components must be able to handle the kilowatt powers necessary to produce sufficient rf field intensity during solid-state NMR experiments. In order to meet these specifications, we have designed a robust, two-part coil holder, illustrated in Fig. 2. Photographs of a fully assembled rotatable flat-coil holder are provided in Fig. 3. This design is portable to a wide variety of probes and magnets; exact dimensions of the various components are not given here because these will be highly dependent on the particular probe being used as well as on the magnet bore diameter. Before going into the design details, it should be noted that the

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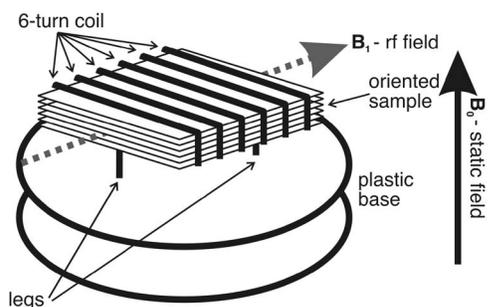


FIG. 1. A schematic diagram of a standard flat coil for static solid-state NMR. The directions of the static magnetic field ( $B_0$ ) of the superconducting magnet of the spectrometer and of the radiofrequency field exerted upon the application of current to the coil or induced by precessing nuclei in the sample ( $B_1$ ) are illustrated.

performance of rectangular coils in the rotatable coil holder was indistinguishable from those in a standard configuration (Fig. 1) and that probe tuning and matching were not adversely affected upon changing to the rotatable holder.

The flat coil is fixed with respect to a hollow, plastic cylinder, with the centerline down the coil (running along the  $B_1$  vector shown in Fig. 1) collinear with the axis of symmetry of the cylinder. This means that rotation around the cylindrical axis of symmetry maintains the direction of  $B_1$ , provided that this axis is perpendicular to  $B_0$ . Neither end of the cylinder is retained during fabrication, which makes the coil easily accessible for sample handling. Note that a slice has been removed during fabrication on a plane parallel to the axis of symmetry lengthwise along the cylinder surface. This provides improved access for soldering or other delicate operations. Also note that the top portion of the cylinder section has narrower diameter walls.

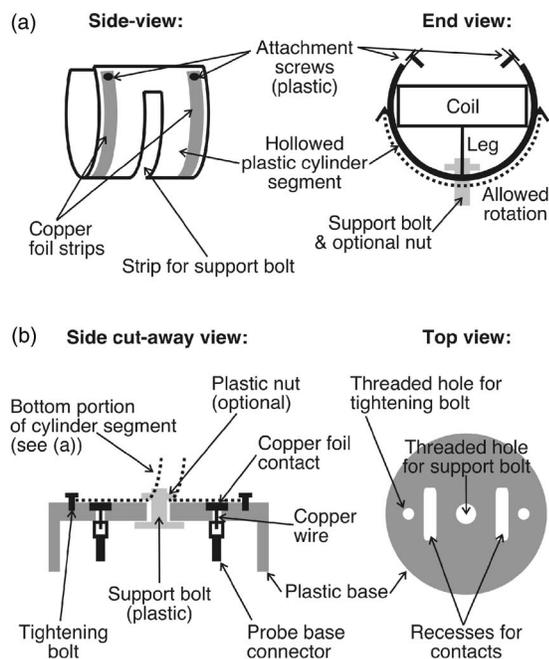


FIG. 2. A schematic illustrating features of a rotatable flat coil holder for static solid-state NMR. (a) Side and cross sectional views of the upper portion. (b) Cutaway and top views of the lower portion. Sections (a) and (b) are attached, anchored to each other at the support bolt, and firmly attached using the tightening bolts. See the main text for a full description.



FIG. 3. (Color online) Photographs of a rotatable flat coil holder built for a Chemmagnetics Apex DRNS Wideline static solid-state NMR probe.

Two strips of copper foil are affixed to the exterior of the cylinder segment at each end, and aligned holes bored through both the plastic and foil allow each leg of the coil to be soldered directly to one of the strips of copper foil. This also means that coils can be easily exchanged, since these joints may be readily de-soldered. Holes at each end of each strip of foil aligned with threaded holes in the cylinder segment allow attachment of the strip directly to the cylinder segment using screws. This is the narrower diameter section of the cylinder wall, which means the screw heads are recessed so as not to restrict rotation. A strip of plastic is cut from the middle of the cylinder segment, running around the exterior, parallel to the two strips of copper, with a width equal to the diameter of the plastic support bolt. This bolt may be tightly attached to the cylinder segment using a plastic nut, as illustrated in Fig. 2, if desired.

The support bolt is threaded through the base, as illustrated in Fig. 2(b). A pair of rectangular copper contacts are soldered to wires and fixed in recesses in the plastic base. These are separated at the same distance as the coil legs and copper strips on the cylinder segment exterior, which have a separation equal to that of the attachment ports in the probe base. Each of the wires is soldered to a connector pin that allows a firm attachment of the entire plastic base and coil holder to the probe. These connectors, their spacing, and the plastic base dimensions are standard for any of the interchangeable coils of our probe. A pair of bolts threaded into holes on the cylinder-long axis just outside the cylinder body allow rotation when loosened and fix the orientation while providing rigidity to the entire assembly when tightened. The previously mentioned nut on the support bolt may be used if additional rigidity is required. We find that the pair of screws at the exterior of the cylinder and the presence of the support bolt in its slot are sufficient to provide a very stable coil holder, however, making this unnecessary. At this point, the coil assembly is complete, and the entire coil holder may be attached to the probe base (Fig. 3).

Although designs were envisioned and built using flexible conductors, we have avoided this in the present holder for two major reasons. First, the use of flat copper contacts serve a dual purpose as support points for the coil holder, adding to its stability. Second, flexible conductors either have to be insulated, or to be very carefully positioned in order to avoid arcing. Insulated conductors able to handle the currents and voltages employed for solid-state NMR will often be too

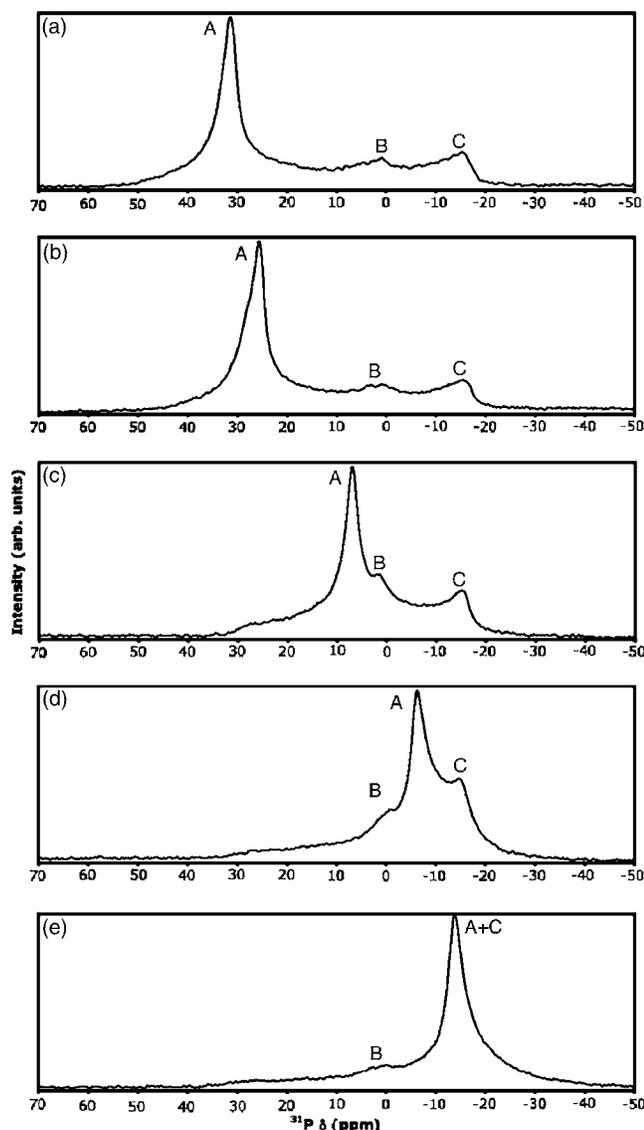


FIG. 4. High-power  $^1\text{H}$ -decoupled (Ref. 9)  $^{31}\text{P}$ -NMR spectra collected at 121.4 MHz using the rotatable flat coil shown in Fig. 3 at rotation angles between the normal to the mica sheet and  $\mathbf{B}_0$  of (a)  $0^\circ$ , (b)  $20^\circ$ , (c)  $45^\circ$ , (d)  $60^\circ$ , and (e)  $90^\circ$ . The sample is 100:1 (molar) POPC to peptide ( $\text{K}_2\text{GL}_{24}\text{K}_2\text{A}$ ) deposited from 3-pentanol onto 24 sheets mica, desolvated, then hydrated with 10 equivalents water and wrapped with plastic food wrap (Ref. 5). Intensities of each spectrum normalized to the most intense point of the spectrum; spectra were aligned at peaks B and C to correct for a chemical shift perturbation arising from the bulk magnetic susceptibility effect (Ref. 10).

stiff to allow easy rotation while arcing will actually occur through the insulation if sufficiently flexible, but subspecification wire is used.

The capabilities of our coil holder are demonstrated with a set of experiments carried out on lipid bilayer samples having some transmembrane peptide incorporated, mostly oriented relative to the plane of the flat substrate (Fig. 4). Sample preparation and data acquisition were as described previously,<sup>5</sup> using 3-pentanol (Aldrich, St. Louis, MO) as the film deposition solvent for a sample of  $\sim 62$  mg 1-palmitoyl-2-oleoyl-*sn*-3-glycerophosphocholine (POPC; Northern Lipids, Vancouver, BC) and  $\sim 5.3$  mg of a chemically synthesized peptide (sequence  $\text{K}_2\text{GL}_{24}\text{K}_2\text{A}$ , free *N*-terminus,

amidated *C*-terminus; synthesized in house) on 24 plates of freshly cleaved muscovite mica (grade V-4; SPI Supplies, West Chester, PA). The holder shown in Fig. 3 was used in these experiments with a flat coil of copper wire (22 AWG, flattened) with inner dimensions  $15.4 \times 15.4 \times 1.5$  mm. All experiments were performed with a Chemmagetics Apex DRNS Wideline static probe (Varian Associates, Fort Collins, CO) in a wide bore superconducting magnet (Oxford Instruments, Oxford, UK) operating on a Unity 300 MHz spectrometer (Varian, Palo Alto, CA). Rotation angles were determined by a protractor and a level. Probe tuning and matching ranges were very similar to both a stationary flat coil (Fig. 1) and to a 10 mm inner diameter, five-turn solenoid coil from the probe manufacturer. Pulse widths, signal intensities, and lineshapes observed were also indistinguishable between the stationary and rotatable flat coils for high-power  $^1\text{H}$ -decoupled  $^{31}\text{P}$  spectra at 121.409 MHz, indicating that the rotatable coil holder is not affecting the Q factor of the probe.

In Fig. 4, we show a series of  $^{31}\text{P}$ -NMR spectra for this oriented bilayer-peptide sample acquired at different rotation angles. The signal arises only from the phosphate group of the POPC lipids. Three spectral components may be resolved. Based on the nature of the system, we attribute these peaks to three different lipid conformations. The major peak (labeled A) corresponds to phospholipid head groups that are in bilayers with their normal oriented parallel to the normal of the substrate. Its chemical shift depends upon the angle ( $\theta$ ) between the bilayer normal and  $\mathbf{B}_0$ .<sup>9</sup>

$$\delta(\theta) = -\frac{2}{3}(\delta_{\perp} - \delta_{\parallel})\left(\frac{3\cos^2\theta - 1}{2}\right) + \delta_{\text{iso}}, \quad (1)$$

where

$$\delta_{\text{iso}} = \frac{1}{3}(2\delta_{\perp} + \delta_{\parallel}), \quad (2)$$

and  $\delta_{\perp}$  and  $\delta_{\parallel}$  are the components of the chemical shift anisotropy tensor for a phosphate group undergoing rotational averaging in the bilayer setting,  $\sim -14.9$  ppm and  $\sim 31.6$  ppm, respectively, in Fig. 4. As we rotate the sample, this peak moves upfield and its position compared to Eq. (1) implies that measured  $\theta$  values have a maximum error of  $\sim 2^\circ - 3^\circ$ . The other two spectral components arise from un-oriented lipids in bilayers, which produce a powder pattern determined by  $\delta_{\perp}$  and  $\delta_{\parallel}$  (peak C), and from lipids in liposome impurities, which rotate rapidly, producing a peak at  $\delta_{\text{iso}}$  [peak B; Eq. (2)]. Peaks arising from each of these types of lipid should remain fixed in position, as observed in Fig. 4. Note that a correction is required to account for orientation-dependent chemical shift changes arising from the bulk magnetic susceptibility (BMS) effect.<sup>10</sup> Therefore, each spectrum was manually adjusted so that peaks B and C are at the same chemical shift as at  $0^\circ$ , with a maximum correction of  $\sim -22.3$  ppm required between  $0^\circ$  and  $90^\circ$ . The position of the downfield edge of the powder pattern (peak C), seen at  $\delta_{\parallel}$  as  $\theta$  increases, confirms the spectral alignment.

All in all, the position and behavior of the three peaks as we vary  $\theta$  implies that the sources of each are correctly assigned and provide a succinct demonstration of the utility and capabilities of our rotatable flat coil holder.

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