# Development of a Triple-resonance SAS Probe with Field Gradient Coil for Dynamic Control over Alignment of Proteins in Bicelles

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## INTRODUCTION

Long-range constraints from residual dipolar couplings in partially aligned solutions have been shown to be very useful in macro-molecule structure determination. Partial macromolecule alignment has been obtained by using dilute liquid crystal solutions of disc-shaped bicelles, but this alone does not permit dynamic control over the alignment.

Switched Angle Spinning (SAS) techniques should provide the needed dynamic control over the bicelle (hence, the protein) alignment. When a sample containing discoidal bicelles of negative magnetic anisotropy is spun at $54.7^0$ with respect to $B_0$ in Magic Angle Spinning (MAS), their interaction with $B_0$ vanishes and their orientation becomes random. For sample spinning at angles less than $54.7^0$, they align with their normals perpendicular to the spinning axis, while spinning at greater angles causes their normals to align with the spinning axis. Dynamic control over the spinning axis is expected to provide the protein alignment control needed for more effective utilization of the bond angle information inherent in the residual dipolar coupling.

A novel SAS probe suitable for such experiments in high-field wide-bore magnets is described here, and a probe suitable for such experiments in narrow-bore magnets is also under development.

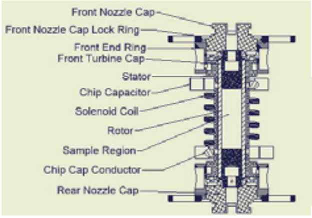
Multi-coil rf circuits are shown to permit greatly improved sensitivity on all channels in the high-field triple-tuned SAS probe, where long leads are unavoidable. ***A low-E $^1$H cross-coil achieves order-of-magnitude reduction in rf decoupler heating compared to multi-tuned solenoids***, and an outer solenoid is used for the mid-frequency (MF) and low-frequency (LF). Efficient H/X/Y performance has been demonstrated with both 4 mm and 7 mm rotors.

Objectives demonstrated in a 4 mm HXY WB SAS probe are:

1. Stable sample spinning over the range of 150 – 14,000 Hz during SAS NMR experiments.
2. Spinning axis reorientation and settling times of 30 ms for angular flips of < 30° for MAS up to 10 kHz.
3. Spinning axis angle control of better than 0.05°.
4. Order-of-magnitude reduction in rf decoupler heating compared to conventional coil designs.
5. Spectral resolution of 0.006 ppm with MAS.
6. RF coil lead-life typically greater than 300,000 22° flips with novel approach to leads.

## High-stability SAS Spinner Designs

Axial symmetry (reflection about the center) has generally been thought to be essential for minimizing axial-force transients during rapid angle re-orientation in SAS NMR. **Figure 1**, below, depicts a longitudinal sectional view of the XC5 (5 mm) or XC4 (4 mm) spinner. Note that it is driven by a radial-inflow microturbine at each end and axially centered by two axial thrust bearings formed from the portion of the radial bearing gas that exits over the ends of the rotor and then radially inward. While its precise axial symmetry and axial bearing at each end have permitted rapid (25 ms) flipping while spinning over 12 kHz with a 5 mm rotor, the presence of the turbine cap and bearing at each end has also made it impossible to implement automatic sample exchange with this spinner.

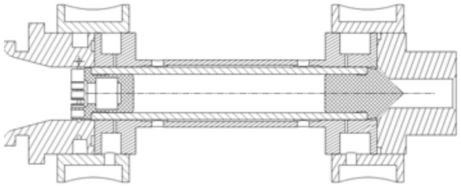



The complexities associated with the servo mechanism at the base of the SAS probe make its installation rather complex. Hence, autosample exchange capability is highly desirable.

A number of drop-in spinner designs were evaluated for compatibility with auto sample exchange. For example, in the Bartuska derivative of the Beams design, a circle of air jets in a conical bearing surface generate a high-velocity, rotating outward flow against the conical, vaned, bottom end (drive tip) of the sample rotor. Here, a stable axial bearing is formed from a balance between Bernoulli and hydrostatic effects under proper conditions. Unfortunately, this approach has inadequate axial stiffness for SAS and exhibits a Bernoulli restoring force only over a very short range of axial displacements, partly because of the rotational flow and its contribution to the total static pressure.

## Development of an Ultra-stable Drop-in (DI-4) Spinner Utilizing a Novel, Inflow Bernoulli Bearing

We recently determined that a high-stiffness Bernoulli/hydrostatic bearing with exceptional stability may be made by reversing the jet orientations and adjusting the surface angles, as shown below in **Figure 2**, with the minimum clearance between the conical surfaces occurring at the minor rather than the major diameters (patent pending). Detailed computational fluid dynamics (CFD) simulations were useful in arriving at an optimum design.



Gas is injected radially inward with a large axial rearward component and a small negative azimuthal component into the conical bearing region from its periphery near the right end in the above drawing. A self-stabilizing axial bearing may be formed with improved stability and stiffness for rotor surface speeds up to at least 80% of the speed of sound. Typical axial oscillations, measured using a linear variable displacement transducer (LVDT), are under 2 $\mu$m rms over a wide range of spinning conditions. ***This is an order of magnitude better axial stability than what has often been seen in alternative spinner designs compatible with automatic sample exchange.***

The primary limitation of this axial bearing is that it cannot provide significant drive without losing the needed Bernoulli suction. That is, the suction (axial) nozzles cannot have a significant tangential component. This is primarily because conservation of angular momentum in the radially inward rotating flow causes tangential flow velocities to increase as the radius decreases, leading to high static pressure, which defeats the Bernoulli effect. So the drive torque must come from a microturbine at the top end. This makes it impractical for the bearing cap and the drive cap to have both the same mass and the same inertia with respect to a re-orientation axis through the center of the rotor. However, by offsetting the re-orientation axis slightly, the axial force transients during flipping can be sufficiently minimized for very fast stable spinning with re-orientation times below 25 ms.

A similar 3 mm spinner (**DI-3**) demonstrated 30 kHz MAS, and the 4 mm spinner **(DI-4)** achieved 23 kHz. With fast flipping SAS (25 ms for a $30^0$ flip), we have achieved 11 kHz spinning with excellent stability.

A specialized adaptation of the 3 mm spinner with a zirconia dewar between the rotor and the rf coils is being used in our CryoMAS probe to permit sample temperatures well above RT while the rf coils are at cryogenic temperatures.

## Building on the Proven XC Advantages

As in most of our solids H/X/Y circuits above 400 MHz, the SAS probe uses a highly optimized cross-coil (XC) for $^1$H and an outer solenoid for the LF and MF channels (see **Figure 3**) because of the strong advantages this approach offers in reducing decoupler heating by an order of magnitude, permitting higher decoupling in high-field MAS, and achieving higher S/N. For example, we have demonstrated S/N greater than 200 on glycine $^{13}$C for H/C/N tuning at 750 MHz - about twice the S/N of alternative solids probes for narrow bore magnets when in the triple configuration.

The dual-coil approach was used in the earlier 600 MHz 7 mm (XC7) WB probe shown in **Figure 4** and in the latest 400 MHz XC4 SAS probe shown in **Figure 5**.

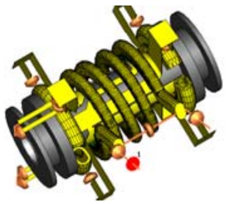


Figure 3. The $^1$H XC and the LF/MF solenoid are shown here as simulated in CST MWS 5.0. The $^1$H capacitors (8 for tuning, plus feed and balance) are represented by chamfered disks.

Figure 4. The LF/MF flex leads used on this earlier 600 MHz H/X/Y SAS probe had to be replaced after ~50,000 flips.

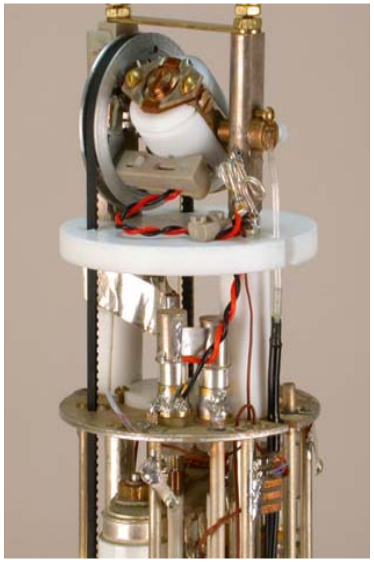


Figure 5. Photo of latest WB SAS probe using an XC4 spinner with novel approaches to rf leads and position feedback and encoding. A fused glass-fiber light pipe is visible coming in along the reorientation axis on the near side for optical spin-rate detection. The MF/LF lead connections on the opposite side are not visible in this view, but are handled in a way similar to commutator connections in some DC motors.

## NMR Results

Angle Setting Reproducibility. A series of experiments that are exceptionally sensitive to angle setting were carried out to determine angle reproducibility during SAS. Deuterated oxalic acid dihydrate was prepared according the method used by Antonijevic and Bodenhausen ([1], and personal communication). A $^2$H wideline experiment was performed using the quad echo pulse sequence, at 46.1 MHz in a 7 T widebore magnet. The 90° splitting from the powder pattern of the crystalline oxalic acid deuterons gives the parameter, $\Delta\nu_0$. This splitting is invariant to rotor angle and was measured as 77960 Hz. The MAS experiment was performed at 5 kHz spinning speed with a rotor synchronized $^2$H experiment where the spectral width = spinning speed (5 kHz) and acquisition delay = 1 rotor cycle (200 $\mu$s). The oxalic deuterons show a residual quadrupolar powder pattern in the isotropic peak, from which can be measured the splitting, $\Delta\nu_1$. The ratio of peak splittings for oxalic acid from static and MAS measurements yields the scaling factor, and is equal to $(3\cos^2\theta-1) / 2$. Experimental data yields $\Delta\theta$, the deviation from magic angle, with sensitivity of several millidegrees.

The housing angle can be intentionally mis-set to a controlled value, whose sign is known, to report the absolute angle of rotation. For these experiments, the rotor angle set by the controller was always $\geq$54.7. First, the splitting was minimized by using a DOTY SAS controller to approach magic angle in steps of 0.05°. Representative spectra are shown in **Figure 6A** and **6B**. Next a SAS experiment was performed by flipping to an angle of 70° and then back to the angle of spectrum 6B, and acquiring. This was done for 256 SAS flips to determine the reproducibility of the housing angle set by the DOTY SAS controller, spectrum shown in **Figure 6C**.

Applications to Bicelles. $^2$H NMR was also performed on $D_2O$ in bicelles prepared with DMPC/DHPC=3.5, 33% by weight in $D_2O$. Samples were spun at 320 Hz at 35 °C in a 40 $\mu$L sealing cell inside a DOTY 4 mm rotor, using a DOTY HXY SAS probe. After spinning at $\theta$=80° (angle between spinning axis and $B_0$) for at least 4 hours, the spectrum in **Figure 7A** was recorded. This splitting corresponds to the bicelle orientation in which the director axis is parallel to the spinning axis and perpendicular to $B_0$ [2]. If the spinning axis is switched to a direction parallel to $B_0$, the bicelles orient in the metastable position that is parallel to $B_0$. The spectrum in **Figure 7B** was acquired after 8 SAS flips from $\theta$=80° to $\theta$=10°. This orientation, of importance for NMR studies on membrane bound proteins, can be obtained in the SAS experiment *without* the use of lanthanides (which may induce paramagnetic shifts and line broadening).

## Rotor-synchronized $^2$H MAS/SAS NMR on Oxalic Acid

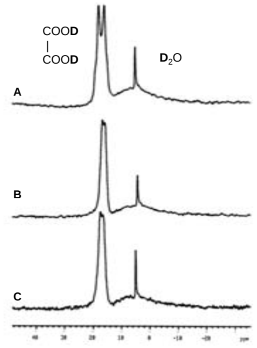



Figure 6. The angle between the spinning axis and $B_0$ is revealed by rotor-synchronized $^2$H MAS/SAS spectra of [$D_6$] oxalic acid dihydrate [1]. The residual quadrupolar powder pattern from the oxalic acid deuterons allows the deviation from the magic angle, $\Delta\theta$, to be estimated as A) $\Delta\theta$=0.049°, B) $\Delta\theta$=0.025° (also serves as reference point for SAS experiment in C), C) spectrum obtained with SAS from $\theta$=70° to $\theta$=magic angle setting determined by spectrum B, nt=256.

## SAS on Bicelles

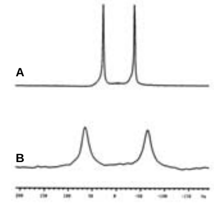



Figure 7. $^2$H quadrupolar splitting for $D_2O$ in bicelles. A) spectrum for sample rotating at $\theta$=80°. B) spectrum on rotating sample obtained with SAS from $\theta$=80° to $\theta$=10°, nt=8.

## CONCLUSIONS

A novel method of angle position feedback appears to permit angle reproducibility of $0.02^0$ in a WB SAS probe, and commutator-type lead contacts permit greatly extended lifetime compared to earlier flex-lead arrangements. These advances may be beneficial in some applications to quadrapolar nuclides. The dynamic control provided by SAS over alignment of bicelles containing proteins may provide more effective utilization of the bond angle information inherent in the residual dipolar coupling. Development is continuing on a similar SAS probe for use in high-field narrow-bore magnets.

## REFERENCES

1. S Antonijevic and G Bodenhausen, "High-Resolution NMR Spectroscopy in Solids by Truly Magic-Angle Spinning," *Angew.Chem. Int. Ed.* 44, (2005) 2935-2938.
2. G Zandomengehi, M Tomaselli, JD van Beek, and BH Meier, "Manipulation of the Director in Bicellar Mesophases by (Variable Angle) Sample Spinning: A New Tool for NMR Spectroscopy," *J. Am. Chem. Soc.*, 123, (2001) 910-913.

**Acknowledgements: This work supported by NIH 5R44EB000152-03.**