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# Bilayer Sample for Fast or Slow Magic Angle Oriented Sample Spinning Solid-State NMR Spectroscopy

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Solid-state NMR spectroscopy provides valuable information on the structure, dynamics, and macroscopic phase properties of membranes. Whereas nuclear interactions such as dipolar, quadrupolar, and chemical shift anisotropies dominate the static NMR spectra of nonordered solid matter, different approaches have been developed to obtain high-resolution solid-state NMR spectra. First, magic angle sample spinning (MAS) of unordered samples results in efficient averaging of nuclear interaction anisotropies.[1] Fast magic angle sample spinning in combination with multidimensional NMR, high magnetic fields, and isotopic labeling have thus provided high-resolution solid-state NMR spectra (e.g. refs 2 and 3). Whereas dipolar couplings and, therefore, distances can be measured accurately with MAS NMR spectroscopy,[2,4] orientational information in complex systems is lost with this technique. A second approach consists of orienting fibers or membranes with respect to the magnetic field direction. Although considerable narrowing of NMR resonances is achieved, the anisotropy of chemical shifts, quadrupolar interactions, or dipolar couplings is maintained. The orientational dependence of these parameters has been used to determine the alignment and structure of membrane polypeptides (reviewed e.g. in ref 5).

More recently, the two approaches have been combined into magic angle-oriented sample spinning (MAOSS). For this approach membranes have been applied onto small circular glass disks that fit inside 7 mm MAS rotors[6] (Figure 1A). The resulting stacks of glass plates have thereafter been subjected to MAS solid-state NMR spectroscopy. Although centrifugal forces limit the spinning speeds applicable, impressive enhancements in line narrowing are observed when compared to static oriented samples, where residual dipolar and chemical shift anisotropies persist due to imperfect sample alignment. On the other hand, broadening of resonances in MAS by low-frequency undulations, which are present in liposomes, are abolished by mechanically supporting oriented membranes.[6]

In this report the MAOSS technique is further explored. In contrast to previous experiments,[6] an experimental setup has been designed in this laboratory that allows the orientation of membranes with their normal perpendicular to the rotor axis.[7] This is achieved by application of bilayer constituents onto thin polyetheretherketone (PEEK) films. It has been shown previously that either PEEK[8] or glass surfaces (e.g. 9) provide suitable supports for orienting membranes. Advantage has been taken of the flexibility of polymeric sheets which allows them to form a continuous spiral of the film that fits inside MAS rotors (Figure 1B). Support of the membrane normal in the direction of centrifugal forces enables application of high spinning speeds and results in a high degree of sample orientation.[10] Here we explore oriented peptide−lipid systems under fast and slow MAS. Our results indicate that narrow

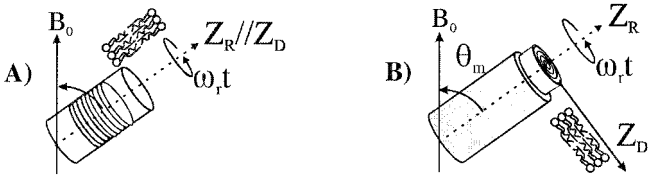


***Figure 1.*** (A) Geometrical arrangement of glass plates and rotor used in conventional MAOSS.[6] (B) Arrangement of the MAS rotor and the membranes, oriented onto a cylinder wrapped from thin PEEK sheets. $Z_D$ = membrane director, $Z_R$ = rotor axis.

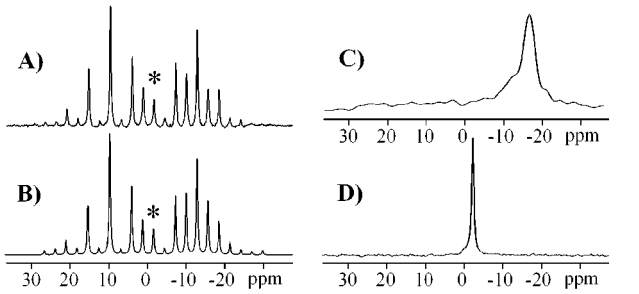


***Figure 2.*** Proton-decoupled Hahn-echo $^{31}$P MAOSS spectra of a lipid-peptide mixture oriented on a cylinder of PEEK film recorded at 300 K under slow ($\omega_r$ = 565 Hz) spinning (A) and its simulation using 80% oriented lipid, mosaic spread of 3.7°, Lorentzian line broadening of 30 Hz, and tensor elements of $\sigma_{||} = 31.9$ ppm and $\sigma_{\perp} = -15.9$ ppm as parameters (B). The asterisk indicates the isotropic resonance. Static $^{31}$P spectrum of the same sample as in (A) with $Z_R||B_0$ (C).$^{31}$P MAOSS spectrum of 10 mg of DMPC lipid bilayers at 10 kHz spinning speed (D).

lines are obtained when at the same time orientational information is maintained (Figure 2A,B).

The theoretical framework for analyzing MAS solid-state NMR spectra has been presented in detail in ref 11. In short, the time-dependent resonance frequency $\omega(t)$ of a particular spin packet in the presence of the chemical shift interaction is expressed with the Wigner rotation matrixes as $D_{q0}$:

$$\omega(t) = \omega_0[\sigma_i + (\sigma_{33} - \sigma_i)D_{00}(\Omega_{PL}) + (\sigma_{11} - \sigma_{22})(D_{-20}(\Omega_{PL}) + D_{20}(\Omega_{PL})]$$

The set of Euler angles $\Omega_{PL}$ describes the transformation from the principal axis system (P) to the laboratory frame (L).[6,12] The spectrum of a sample is calculated by summation of the orientation-dependent signals. The geometrical arrangement of membrane-coated PEEK cylinders in a MAS rotor (Figure 1B) is consistent with a circular distribution of molecules around the rotor axis and fast rotational diffusion of the lipids around the membrane director $Z_D$ (axis system D). The rotor is inclined at the magic angle $\theta_m$ = 54.7° with respect to the magnetic field direction ($B_0$) and spinning at $\omega_r$ about the rotor axis $Z_R$. The transformation from (P) to (L) is thus described by three steps or sets of Euler angles ($\alpha_{PD}$, $\beta_{PD}$, $\gamma_{PD}$), ($\alpha_{DR}$, 90°, $\gamma_{DR}$), and ($\alpha_{RL}$, $\theta_m$, $\omega_r(t)$) (Figures 1B and 3C). A

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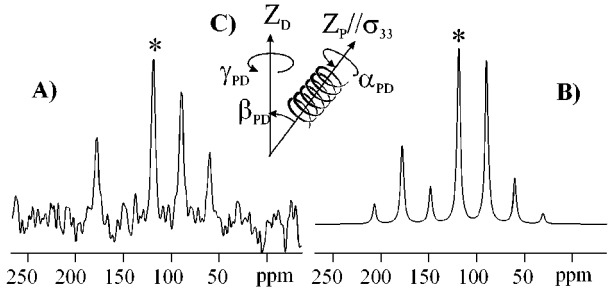


***Figure 3.*** (A) $^1$H-decoupled $^{15}$N cross polarization of the MAOSS NMR spectrum of 4% [$^{15}$N-Leu14]-KKKAL LALLA LWALL LALLA KKK-amide in 80 mg of oriented DMPC. (The asterisk indicates isotropic resonance at 120 ppm, $\omega_r = 1.5$ kHz, 250 K.) $^{15}NH_4Cl$ is used as an external reference (41.5 ppm). (B) Simulation of spectrum A with $\beta_{PD} = 10°$, a Gaussian distribution of 25°, Lorentzian line broadening of 150 Hz, and tensor elements of $\sigma_{11} = 57$ ppm, $\sigma_{22} = 83$ ppm, and $\sigma_{33} = 220$ ppm. (C) Transformation from the Principal Axis System to the membrane director frame.

Gaussian distribution accounts for the mosaic spread of the membrane (Figure 1B), as well as for the distribution of the tilt angle of the peptide (Figure 3C). The orientation of the $^{15}$N chemical shift PAS system (Figure 3C) with respect to (D) is given by the polar angle $\beta_{PD}$. The azimuthal angles $\gamma_{DR}$ and $\gamma_{PD}$ and the polar angle $\alpha_{DR}$ are assumed to be equally distributed.

The sample was prepared by dissolving DMPC (dimyristoyl-*sn*-glycero-3-phosphocholine, Avanti Polar Lipids, Birmingham, AL) and a hydrophobic peptide (prepared by fmoc solid-phase chemistry) with a peptide-to-lipid ratio of 4 mol % in trifluoroethanol. The solution was evenly spread on a film of PEEK (250 × 12 mm, thickness 25 $\mu$m, Goodfellow, Cambridge, UK). After the solvent was evaporated, the sample equilibrated in 93% relative humidity. The film was wrapped into a cylinder of approximately 30 turns and transferred into a 4 mm MAS rotor with ca. 0.5 mm of empty space remaining in the center (Figure 1B). The NMR experiments were carried out on Bruker DSX500 or DSX400 NMR spectrometers. Typical acquisition parameters for $^{31}$P were Hahn-echo pulse sequence at 202.41 (161.97) MHz, 2.2 (3.5) $\mu$s 90° pulse length, 25 kHz spectral width, 27 ms acquisition time, 15−20 kHz CW $^1$H decoupling, and 16−64 transients; those for $^{15}$N were 50.67 MHz, 1 ms MOIST spin lock,[13] 50 kHz spectral width, 8 ms acquisition time, 35 kHz $^1$H decoupling, and 6000 transients.

The samples were inserted into multichannel commercial MAS solid-state NMR probe heads. Importantly, the sample setup with the membrane normal being oriented parallel to the direction of centrifugal forces (Figure 1B) allows for fast magic angle spinning, thereby efficiently averaging chemical shift and dipolar anisotropies. Moreover the sample fits inside a 4 mm rotor. Thus spinning speeds up to 10 kHz have been achieved and more might be possible (Figure 2D), compared to a 3−5 kHz limit with the conventional MAOSS technique.[6] At fast spinning frequencies the number and intensities of spinning sidebands (ssbs) are reduced, and therefore the potential overlap of resonances, which often complicates spectral analysis in more complex systems, is avoided. Only a small amount of material (<5%) leaves the mechanical support at the edges of the polymer support during spinning. Moreover, these powder pattern contributions are far from the sensitive volume of the coil and, therefore, are not detected. This also contrasts the original MAOSS sample setup where material, that escapes from between the glass plates, is located within the coil.[6] Centrifugal forces slightly improve sample alignment (mosaic spread) for this particular geometry.[10] Samples remained stable even during ≤24 h of fast spinning.

At lower spinning speeds, however, sidebands provide valuable information about the alignment of molecules with respect to the rotor axis, and hence about the membrane normal. The experimental $^{31}$P MAOSS NMR ssb pattern recorded at $\omega_r = 565$ Hz with the lipid−peptide mixture is shown in Figure 2A. The intensities of the ssbs of this oriented sample are significantly different from those observed in the presence of powders, some of the ssbs being virtually missing. Excellent agreement between the simulated and the experimental intensities exists when a mosaic spread of 3.7° is taken into consideration (Figure 2A,B). The orientational degree of the sample was tested by transferring it into a static NMR probe head[9] (Figure 2C). The resonance at −17 ppm is indicative of the membrane director being perpendicular to $B_0$. With pure lipids, the percentage of randomly oriented lipid is <10%. Magic angle spinning of this sample also results in efficient averaging of residual anisotropic interactions and lines considerably sharper ($\Delta\nu_{1/2} = 30$ Hz) than those reported previously in conventional MAOSS experiments.[6]

The new technique was also used to investigate the membrane tilt angle of an α-helical peptide. The $^1$H-decoupled $^{15}$N MAOSS spectrum is shown in Figure 3A. The simulated sideband intensities (Figure 3B) were calculated with the geometry described in Figure 3C. The ssb pattern of the sample is indicative of the transmembrane alignment of the peptide α-helix and in agreement with results obtained in static oriented samples (C. Aisenbrey and B. Bechinger, unpublished result).

Whereas previous experiments have demonstrated that narrow lines and high resolution are possible with use of magic angle oriented sample spinning at low spinning speeds,[6] this paper introduces new methodologies that allow this technique to be extended to high MAS frequencies, together with the possibility of using small diameter rotors (e.g. 4 mm). Application of high-resolution multidimensional NMR pulse sequences to fast spinning of oriented membranes is straightforward. At the same time topological and orientational information remains accessible.

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