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Supplement to chapter 20
MAS NMR of the Chlorosomes (I. de Boer, H. J.M. de Groot)

MAS NMR of the chlorosomes (web appendix)

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A. Fast Magic Angle Spinning NMR in high magnetic field

Magic Angle Spinning (MAS) NMR structure determination of multispin labeled solids critically depends on both the resolution and stability that can be achieved with the NMR spectrometer. In both aspects, important improvements have been realized in the past few years. The resolution was improved by fast MAS, high field and modern pulse technology. In particular, the recent realization of ultra high field spectrometer technology has boosted the development and implementation of novel structure determination methodology. The stability required to perform the new generation of experiments was achieved by improvements in the spectrometer electronics hardware, allowing fast, precise and coherent phase and frequency switching during the NMR experiment.

To describe the NMR, the Hamiltonian

$$H_{\mathrm{S}} = H_{\mathrm{CS}} + H_{\mathrm{II}} + H_{\mathrm{IS}} \tag{1}$$

is used (Mehring, 1983). H_{CS} is the chemical shift term, H_{II} contains the homonuclear dipolar couplings and H_{IS} represents the heteronuclear dipolar couplings. Anisotropic spin interactions that are averaged to an isotropic part in liquids cause large NMR line broadening in solids. These are primarily the anisotropic part of the chemical shift and the dipolar spin-spin couplings. The chemical shift σ, for example, depends on the orientation of the chemical environment of the nucleus in the magnetic field.

The anisotropic interactions in solids can be eliminated by fast rotation of the sample around an axis at the magic angle $\theta_{\mathrm{m}} \approx 54.74°$ with the static field . For instance, for the chemical shift it can be shown that the time dependent transformation from the principal axis frame to the laboratory frame leads to a time average of

$$\langle H_{CS} \rangle = (\sigma_{\mathrm{zz}} + 2\sigma_{\mathrm{iso}})\tfrac{1}{2}(3\cos^2\theta - 1)B_0 I_{\mathrm{z}} + \sigma_{\mathrm{iso}} B_0 I_{\mathrm{z}}\,, \tag{2}$$

where σ_{zz} is a tensor element, σ_{iso} is the isotropic component of σ, B_0 is the magnetic field strength, θ is the angle between the rotor axis and $\boldsymbol{B}_0$ and I_z is a spin operator. The first term equals zero for $\theta = \theta_m$ and only the isotropic term survives.

For MAS to be efficient, the spinning rate ω_r needs to be much larger than the strength of the anisotropic interactions. The chemical shift scales with B_0 (see Eq.[2]), while the dipolar couplings are field-independent. Thus, higher magnetic fields are generally desirable as the dispersion of the signals increases relative to the broadening effects of the dipolar couplings and the resolution is enhanced. In addition, high fields increase the sensitivity of the NMR experiments. Since the chemical shift anisotropy also scales with B_0, fast MAS is necessary in high fields. With developments in instrumentation, MAS rates of ~15 kHz are now common practice and higher rates up to ~50 kHz are possible, although at the expense of smaller sample volumes. This is sufficient to reduce the chemical shift anisotropy as well as the homonuclear dipolar couplings between isotope labels such as ^{13}C.

Many of the standard cross polarization (CP) experiments can be applied or have been adopted for use with rapid MAS in high field. The CP technique exploits the high abundance, high sensitivity and short relaxation times of the protons by transferring transverse ^{1}H magnetization to another spin species (Hartmann and Hahn, 1962). The maximum enhancement for a ^{13}C signal compared to direct ^{13}C excitation is $g_{1H}/g_{13C} \approx 4$. In addition, the recycle delay required for the accumulation of the free induction decays is usually short. Overall, CP introduces a significant gain in sensitivity. During the detection of the signal, heteronuclear decoupling is applied to achieve a high resolution. The robust TPPM sequence is now widely used for this purpose (Bennett et al., 1995). It uses 180° pulses with alternating phases for efficient decoupling. The CP/MAS experiment with TPPM decoupling is the building block for more advanced techniques, such as two-dimensional correlation spectroscopy.

B. Homonuclear correlation spectroscopy

To resolve signals and for *de novo* structure determination of solids, correlation NMR spectroscopy of multi-spin labeled molecules is necessary. Here the homonuclear dipolar couplings, that are averaged by MAS to achieve a high resolution, are reintroduced during a mixing interval to generate correlated spin states (Mehring, 1983; Ernst et al., 1987). Two experiments that are widely used and important for the study of organic compounds are ^{13}C-^{13}C and ^{1}H-^{13}C correlation spectroscopy. The sequence of the ^{13}C-^{13}C Radio Frequency-Driven Dipolar Recoupling (RFDR) MAS correlation experiment (Bennett et al., 1992) is shown in Fig. S1A. The preparation step consists of the CP procedure. During t_1 and t_2, the ^{13}C spins precess under heteronuclear decoupling to give a high resolution. During τ_m, however, the dipolar ^{13}C-^{13}C couplings have to be reintroduced to promote transfer of magnetization. The magnetization is first stored along z by a 90° pulse. The actual recoupling is achieved by a series of 180° pulses, which are synchronized with the rotor frequency. The evolution of the spin state ρ is determined by the commutator

$$\frac{d\rho(t)}{dt} = -i\left[\tilde{H}_{\mathrm{II}}, \rho(t)\right], \tag{3}$$

where $\tilde{H}_{\mathrm{II}}$ is a spin Hamiltonian describing the effective reintroduced homonuclear dipolar interactions. This yields

$$\rho(t) = I_{1z}\cos^2(\tau_\mathrm{m} d/2) + I_{2z}\sin^2(\tau_\mathrm{m} d/2) + \tfrac{1}{2}(I_{1y}I_{2x} - I_{1x}I_{2y})\sin(\tau_\mathrm{m} d), \tag{4}$$

where d depends on the dipolar coupling strength (Bennett et al., 1992). The first two terms indicate transfer of longitudinal magnetization, while the third term represents double quantum states, which can be eliminated by phase cycling. If a short mixing time τ_m of ~1 ms is used, only correlations between spins separated by one bond in an organic molecule are created, which is most beneficial for the assignment of the chemical shifts.

To resolve intermolecular arrangements, incoherent processes over longer distances can be used to obtain distance constraints. For instance, the RFDR sequence with a long mixing time has provided ^{13}C-^{13}C correlations spanning larger distances (Boender et al., 1995). Intermolecular transfer between ^{13}C spins, however, is generally difficult for uniformly labeled chlorophylls, due to rapid relayed spin diffusion along the multispin ^{13}C-labeled molecular network. An attractive alternative is to generate ^{13}C-^{13}C correlations by ^{1}H spin diffusion (Mulder et al., 1998; Wilhelm et al., 1998). In this CP^3 experiment the ^{13}C magnetization is transferred back to ^{1}H after the first precession interval. Next, ^{1}H spin diffusion is allowed to take place during a mixing period. Finally, the signal is transferred again to ^{13}C by a third CP step and detected. In this way, mixing by the strong ^{1}H dipolar interactions is combined with the high resolution of ^{13}C. The diffusion experiment was used to determine a maximum effective transfer range d_{max} for short mixing times (de Boer et al., 2002). In this way intermolecular distance constraints were resolved. In practice, a limited number of constraints can already be very useful for elucidating the structure of ordered solids.

C. Heteronuclear correlation spectroscopy

A straightforward ^{1}H-^{13}C correlation experiment consists of the CP scheme, where t_1 is inserted after the first ^{1}H 90° pulse and the CP interval constitutes the mixing step. This is known as Wideline Separation (WISE), since broad ^{1}H lines in the indirect dimension are separated by correlation with ^{13}C shifts in the direct dimension. The homonuclear dipolar couplings between the abundant ^{1}H spins are too strong to be eliminated, even in case of fast MAS.

Although ^{1}H signals can be assigned with the WISE technique, the ^{1}H resolution can be improved considerably if there is little inhomogeneous line broadening. In particular, the robust *Lee Goldburg* (LG) technique employs off-resonance rf irradiation to generate an effective rf field inclined at the magic angle (Lee and Goldburg, 1965; Bielecki et al., 1989). Precession of the spins around this field amounts to “magic angle spinning in spin space”. With the 2D LG/MAS experiment (Fig.S1B), spectra can be obtained with a good resolution in both dimensions (van Rossum et al., 1997). A recent version uses phase modulated *Lee Goldburg* (PMLG), which is easy to implement compared to the frequency switched LG (Vinogradov et al., 1999).

Heteronuclear distances were measured with the cross polarization and *Lee Goldburg* decoupling applied during the CP step to suppress ^{1}H spin diffusion (van Rossum et al., 2000). As a result, the Hamiltonian for a coupled ^{1}H-^{13}C spin pair during LG-CP simplifies to

$$\tilde{H}_{\mathrm{IS}} = \frac{\delta}{4}\left[I_+S_- \exp(i\varphi) + I_-S_+ \exp(-i\varphi)\right], \tag{5}$$

where $I_\pm$ and $S_\pm$ are spin operators for the ^{1}H and ^{13}C spin, respectively. The dipolar coupling δ is given by

$$\delta = -G_1 \sin\theta_{\mathrm{m}} \frac{\mu_0}{4\pi}\frac{\gamma_{\mathrm{I}}\gamma_{\mathrm{S}}\hbar^2}{r_{\mathrm{IS}}^3}\ , \tag{6}$$

with G_1 a geometrical factor and r_{IS} the distance between the spins. The coherent build-up of the ^{13}C signal $S(t)$ is then described by

$$S(t) = -\tfrac{1}{4}(Zk_{\mathrm{B}}T)^{-1}\omega_{0\mathrm{I}}\left(1-\cos\tfrac{1}{2}\delta t\right). \tag{7}$$

From the build-up of $S(t)$ the dipolar coupling can be determined. This technique yields accurate distances up to a few Å. As the dipolar couplings scale with r^{-3}, the effects of long-distance interactions are obscured by strong short-range interactions. For longer CP times, the magnetization transfer is incoherent due to the many spin interactions and relaxation. Although accurate intermolecular distances are difficult to determine in chlorophylls, incoherent long-range transfer proceeds over an effective maximum transfer range d_{max}, which depends on the length of the mixing period (van Rossum et al., 2002).

Finally, dilution of the ^{1}H spins by ^{2}H has been explored to reduce the ^{1}H line widths of chlorophylls (Schulten et al., 1998). Obviously, the reduction in sensitivity is a major disadvantage of this method. With a dilution level of 75%, the ^{1}H line widths in a 2D WISE experiment are narrowed significantly for several signals, although for the aliphatic protons there is little improvement. A 2D FSLG or PMLG experiment outperforms the 75% ^{2}H dilution, since a better resolution is obtained without sacrifying sensitivity.

II. Chemical shift data of ^{13}C enriched Chl *a*/H_2O and chlorosomal BChl *c*

In Table S1 and S2, ^{1}H and ^{13}C chemical shift data is listed that were acquired by MAS NMR of solid aggregated [U-^{13}C]-Chl ***a***/H_2O (Table S1) and [U-^{13}C]-BChl ***c*** in the chlorosomes of *Chlorobium tepidum* (Table S2). A detailed account of these studies is given in the book chapter corresponding to this appendix. See also the references in the figure captions.

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Table S1. ^{13}C and ^{1}H chemical shifts, referenced to TMS ($\delta^{C,\,H}$) and aggregation shifts[a] ($\Delta\delta^{C,\,H}$) for Chl ***a***/H_2O. The estimated errors for the solid state shifts are given in parenthesis.

Position	δ^C (ppm)	$\Delta\delta^C$ (ppm)	δ^H (ppm)	$\Delta\delta^H$ (ppm)
12^1	10.5 (0.3)	-2.1	-1.3 (0.3)	-4.9
2^1	10.2 (0.2)	-2.4	-2.1 (0.3)	-5.4
7^1	8.9 / 11.5 (0.2)	-2.3 / 0.3	1.7 / 2.8 (0.3)	-1.6 / -0.5
P17	17.4 (0.1)	1.2	1.5 (0.5)	0.0
8^2	16.5 / 17.5 (0.5)	-1.5 / -0.5	1 (1)	-0.7
8^1	19.0 / 20.5 (0.5)	-1.0 / 0.5	2 (2)	-2
P18	20.0 (0.2)	0.0	0.9 (0.5)	0.1
P19	20.0 (0.2)	0.0	0.9 (0.5)	0.1
P20	23.3 (0.3)	0.3	1 (1)	0.2
P16	23.3 (0.3)	0.3	1 (1)	0.2
18^1	24.9 (0.1)	1.0	1.4 (0.5)	-0.4
P9	25.6 (0.3)	0.4	1.2 (0.5)	0.0
P13	25.6 (0.3)	0.0	1.2 (0.5)	0.0
P5	25.8 (0.2)	0.0	1.2 (0.5)	0.0
P15	28.4 (0.2)	-0.3	1.3 (0.5)	-0.2
17^1	32.3 (0.1)	2.2 / 1.3 [b]	3.0 (0.5)	0.5
17^2	32.3 (0.1)	2.2 / 1.3 [b]	3.0 (0.5)	1.0
P7	33.1 (0.1)	-0.3	1.2 (0.5)	-0.1
P11	33.1 (0.1)	-0.5	1.2 (0.5)	-0.1
P6	37.1 (0.5)	-0.3	1.0 (0.5)	-0.1
P12	38.0 (0.2)	0.0	1.1 (0.5)	0.0
P8	38.0 (0.2)	0.0	1.1 (0.5)	0.0
P10	38.0 (0.2)	0.0	1.1 (0.5)	0.0
P14	40.0 (0.2)	-0.1	2.0 (0.5)	0.9
P4	40.0 (0.2)	-0.4	2.0 (0.5)	0.2

18	50.0 (0.1)	0.0	2.8 (0.5)	-1.8
17	51.7 (0.3)	0.1	3.9 (0.5)	-0.3
13^4	51.8 (0.1)	-0.2	3.4 (0.5)	-0.4
P1	61.7 (0.1)	0.4	4.9 (0.3)	0.6
13^2	64.9 (0.1)	-1.3	5.8 (0.5)	-0.4
20	92.6 (0.2)	-0.2	6.7 (0.3)	-1.9
5	98.3 (0.1)	-1.7	8.6 (0.3)	-0.8
15	103.0 (0.1)	-3.2		
10	108.1 (0.1)	1.0	7.8 (0.3)	-1.9
3^2	113.7 (0.1)	-5.2	2.3 (0.5)	-3.9 / -3.7
P2	121.3 (0.1)	1.9	6.0 (0.3)	1.0
13	126.1 (0.1)	-5.4		
3^1	126.7 (0.1)	-4.8	5.7 (0.3)	-2.4
7	132.2 / 133.3 (0.1)	-1.8/-0.7		
12	133.0 (0.1)	-1.2		
2	135.6 (0.1)	0.1		
3	136.3 (0.1)	-2.7		
P3	138.5 (0.1)	-3.7		
8	145.4 (0.1)	1.3		
9/11	147.0 (0.1)	0.9/-0.7 [b]		
4	150.7 (0.1)	2.7		
6	154.1 (0.2)	2.7		
16	154.0 (0.1)	-1.8		
1	156.1 (0.1)	2.1		
14	162.3 (0.1)	0.9		
19	169.4 (0.3)	2.0		
13^3	169.9 (0.1)	-1.1		
17^3	174.3 (0.3)	1.6		
13^1	190.6 (0.1)	1.3		

[a] Calculated by substracting the corresponding ^{1}H and ^{13}C shifts for monomeric Chl ***a*** in acetone-d_6, which were obtained from Van Rossum (2002) and sources cited therein.

[b] The differences between 9-C/11-C, and between 17^1-C/17^2-C are not resolved in solution.

Table S2. ^{13}C and ^{1}H chemical shifts, referenced to TMS ($\delta^{C, H}$) and aggregation shifts ($\Delta\delta^{C, H}$) for BChl ***c***. The estimated errors for the solid state shifts are given in parenthesis.

Position	δ^{C} (ppm)[a]	$\Delta\delta^{C}$ (ppm)[a]	δ^{H} (ppm)[b]	$\Delta\delta^{H}$ (ppm)[c]
7^1	10.4 / 6.6 (0.3)	0 / -4.2	3.1 / -0.5 (0.3)	0 / -3.7
F7^1	16 (1)	0	1.2 (0.6)	0
F3^1	16 (1)	0	1.2 (0.6)	0
12^2	16.8 (0.6)	0	2 (2)	0
2^1	13.8 (0.7)	-2.5	1.1 (0.3)	-2.2
8^2	16.9 (0.6)	-0.5	2 (2)	0
F11^1	16.6 (0.6)	-0.5	1.5 (1)	0
8^1	19 (1)	0	2 (2)	-2
18^1	21 (1)	-1	2 (1)	0
12^1	17.5 (0.6)	-3.5	2 (1)	-2
20^1	20 (1)	-1	3 (1)	-1
F12	25.2 (0.3)	0	1.2 (0.6)	0
3^2	22.7 (0.3)	-3	1.0 (0.6)	-1
F9	25.7 (0.4)	0	2.0 (0.3)	0
F5	25.7 (0.4)	0	2.0 (0.3)	0
17^1	30 (2)	0	1.4 (0.3)	-0.7
17^2	30 (2)	0	1.4 (0.3)	-1.0
F8	39.1 (0.3)	0	1.8 (0.3)	0
F4	39.1 (0.3)	0	1.8 (0.3)	0
18	48.1 (0.4)	0	4 (1)	-1
13^2	48.1 (0.4)	0	4.5 (0.6)	-0.6
17	49.7 (0.4)	0	4 (1)	0
F1	60.9 (0.3)	0	4.5 (0.3)	0
3^1	63.7 (0.7)	-1	3.0 (0.5)	-3.3
5	101.2 / 95.1 (0.3)	-4.5	7.5 / 8.4 (0.3)	-2.1 / -1.2

20	105.0 / 103.5 (0.3)	0		
15	104.3 (0.7)	-0.5		
10	105.5 (0.5)	0	10.0 (0.3)	0.6
F2	119.1 (0.3)	1.5	5.4 (0.3)	0
F6	124 (1)	0	5.0 (0.3)	0
F10	124.0 (1.0)	0	5.0 (0.3)	0
13	127.9 (0.5)	-2.5		
F11	130.2 (0.3)	-1		
7	132.3 / 131.3 (0.3)	-1.5 / -2.5		
F7	135 (1)	0		
2	135.2 (0.3)	0		
12	139 (1)	-2		
F3	140.1 (0.3)	-1.5		
8	142.1 / 140.1 (0.3)	0 / -2.5		
3	139 (1)	-5		
4	143.5 / 144.4 (0.3)	-1.8 / -0.9		
9	146.1 / 146.8 (0.3)	0, 0		
11	146.8 (0.4)	0		
6	149.8 / 150.5 (0.3)	-0.8 / 0		
1	153.2 (0.5)	0		
16	153.6 (0.5)	0		
14	162.1 (0.3)	-1		
19	169.0 / 166.7 (0.3)	1.2 / -1.1		
17^3	173.0 (0.5)	-1		
13^1	195.8 (0.3)	-2.5		

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[a] Values taken from Balaban (1995) and Van Rossum (2001) for the doubled signals. [b] Values taken from Van Rossum (2001). [c] Calculated from the data given in Van Rossum (2001).

Figure caption

Fig. S1. (A) Pulse sequence of the 2D ^{13}C-^{13}C MAS NMR radio frequency-driven dipolar recoupling homonuclear correlation experiment. Following a cross polarization (CP) step, the ^{13}C spins precess freely during t_1, while heteronuclear decoupling (DEC) is applied on the ^{1}H spins. Subsequently, the ^{13}C-^{13}C couplings averaged by MAS are reintroduced by a rotor-synchronized train of 180° pulses. During t_2 the ^{13}C free induction decay is detected. (B) Pulse sequence of the 2D ^{1}H-^{13}C MAS NMR LG-CP heteronuclear correlation experiment. After ^{1}H excitation, the ^{1}H spins precess under homonuclear Lee-Goldburg (LG) decoupling during t_1. Subsequently, a CP step forms the mixing interval and the ^{13}C free induction decay is detected in t_2.