

## Probing Segmental Order in Lipid Bilayers at Variable Hydration Levels by Amplitude- and Phase-Modulated Cross-Polarization NMR

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### A. THE APM-CP TECHNIQUE

A schematic pulse sequence for 2D SLF spectroscopy using amplitude- and phase-modulated cross-polarization (APM-CP) during the variable evolution period  $t_1$  is shown in Fig. 1a in the communication. It starts by ramped CP signal enhancement to prepare spin-locked magnetizations. The dipolar evolution period is initiated by inverting the phase of the  $^1\text{H}$  spin-lock field. The heteronuclear dipolar couplings are monitored through the oscillations resulting from the coherent polarization transfer between  $^1\text{H}$  and  $^{13}\text{C}$  spins during  $t_1$ . To suppress rf mismatch and inhomogeneity effects, the CP fields during  $t_1$  are phase modulated.

Under MAS with a spinning frequency  $\omega_r$ , the heteronuclear  $^1\text{H}$ - $^{13}\text{C}$  dipolar coupling is most efficiently maintained by matching the two rf fields to the first order spinning sidebands  $\omega_{1,\text{H}} - \omega_{1,\text{C}} = \gamma_{\text{H}}B_{1,\text{H}} - \gamma_{\text{C}}B_{1,\text{C}} = n\omega_r$  with  $n = \pm 1$ . To avoid cancellation of the recoupling effect by the periodic and fast (on the time scale of the rotor period) rf phase inversion, alternation of the Hartmann-Hahn (HH) matching condition between  $n = +1$  and  $n = -1$  sidebands synchronized with the phase switching is employed in analogy with amplitude-modulated FSLG-CP.<sup>1</sup>

The interaction frame dipolar Hamiltonian for an isolated  $I$ - $S$  spin pair during CP with continuous rf fields is given by

$$\begin{aligned} \tilde{H}_{IS}^{T,MAS}(t) = & \\ & \sum_{m=\pm 1, \pm 2} \frac{\omega_{IS}^{(m)}}{2} \left\{ \left[ e^{i(\Delta\omega + m\omega_r)t} + e^{-i(\Delta\omega - m\omega_r)t} \right] ZQ_x + \left[ e^{i(\Delta\omega + m\omega_r)t} - e^{-i(\Delta\omega - m\omega_r)t} \right] iZQ_y \right\}, \end{aligned} \quad (1)$$

where  $\Delta\omega = \omega_{1,I} - \omega_{1,S}$ ,  $ZQ_x = [I_+S_- + I_-S_+]/2$ ,  $ZQ_y = -i[I_+S_- - I_-S_+]/2$ , and double-quantum terms are neglected. The Fourier components of the dipolar coupling constant are given by

$\omega_{IS}^{(m)} = b^{(m)} e^{\pm im\gamma}$ , where  $b^{(\pm 1)} = [b/(2\sqrt{2})] \times \sin(2\beta)$ ,  $b^{(\pm 2)} = (b/4) \times \sin^2\beta$ ,  $b = -(\mu_0/4\pi)(\gamma_I \gamma_S \hbar / r^3)$ , and the angles  $\beta$  and  $\gamma$  describe the orientation of the internuclear vector with respect to the rotor frame.<sup>2</sup> At sideband HH matching,  $\Delta\omega - n\omega_r = 0$ , the secular part of the dipolar Hamiltonian takes the form

$$\widetilde{H}_{IS}^{T,MAS} = b^{(n)} [\cos(n\gamma)ZQ_x + \sin(n\gamma)ZQ_y]. \quad (2)$$

For a sequence containing two CP pulses of durations  $\tau_1$  and  $\tau_2$  with nutation frequencies of  $\omega_{1,I(1)}$ ,  $\omega_{1,S(1)}$  and  $\omega_{1,I(2)}$ ,  $\omega_{1,S(2)}$ , respectively, the interaction frame Hamiltonian can be calculated using the rf propagators

$$U_{rf(1)}(t) = \exp(-i\omega_{1,I(1)}tI_z - i\omega_{1,S(1)}tS_z), \quad \text{for } 0 \leq t \leq \tau_1 \quad (3a)$$

$$U_{rf(2)}(t) = \exp[i\omega_{1,I(2)}(t - \tau_1)I_z + i\omega_{1,S(2)}(t - \tau_1)S_z] \times \exp(-i\omega_{1,I(1)}\tau_1 I_z - i\omega_{1,S(1)}\tau_1 S_z), \quad \text{for } \tau_1 < t \leq \tau_1 + \tau_2 \quad (3b)$$

The average, over a cycle time of  $\tau_c = \tau_1 + \tau_2$ , Hamiltonian is given by

$$\begin{aligned} \widetilde{H}_{IS}^{T,MAS}(\tau_c) = & \\ \frac{1}{\tau_c} \sum_m \frac{\omega_{IS}^{(m)}}{2} & \left[ \left( \frac{e^{i(\Delta\omega_{(1)} + m\omega_r)\tau_1} - 1}{i(\Delta\omega_{(1)} + m\omega_r)} - \frac{e^{i(\Delta\omega_{(1)} + m\omega_r)\tau_1 + i(-\Delta\omega_{(2)} + m\omega_r)\tau_2} - e^{i(\Delta\omega_{(1)} + m\omega_r)\tau_1}}{i(\Delta\omega_{(2)} - m\omega_r)} \right) (ZQ_x + iZQ_y) - \right. \\ & \left. \left( \frac{e^{-i(\Delta\omega_{(1)} - m\omega_r)\tau_1} - 1}{i(\Delta\omega_{(1)} - m\omega_r)} - \frac{e^{-i(\Delta\omega_{(1)} - m\omega_r)\tau_1 - i(-\Delta\omega_{(2)} - m\omega_r)\tau_2} - e^{-i(\Delta\omega_{(1)} - m\omega_r)\tau_1}}{i(\Delta\omega_{(2)} + m\omega_r)} \right) (ZQ_x - iZQ_y) \right], \end{aligned} \quad (4)$$

which, under the condition

$$\Delta\omega_{(1)} = -\Delta\omega_{(2)} \equiv \Delta\omega, \quad (5)$$

simplifies to

$$\widetilde{H}_{IS}^{T,MAS}(\tau_c) = \frac{1}{\tau_c} \sum_m \frac{\omega_{IS}^{(m)}}{2} \left[ \frac{e^{i(\Delta\omega + m\omega_r)\tau_c} - 1}{i(\Delta\omega + m\omega_r)} (ZQ_x + iZQ_y) - \frac{e^{-i(\Delta\omega - m\omega_r)\tau_c} - 1}{i(\Delta\omega - m\omega_r)} (ZQ_x - iZQ_y) \right]. \quad (6)$$

An analogous expression is obtained if several CP cycles are applied during the rotor period  $T$  provided that the condition in Eq. (5) is satisfied. At HH sideband matching, the following average Hamiltonian is obtained

$$\widetilde{H}_{IS}^{T,MAS}(T) = b^{(n)} [\cos(n\gamma)ZQ_x + \sin(n\gamma)ZQ_y], \quad (7)$$

which is independent of the number of cycles per rotor period and coincides with Eq. (2) at multiples of  $T$ .

To satisfy both Eq. (5) and the HH condition  $\Delta\omega - n\omega_r = 0$ , the nutation frequencies are set to

$$\omega_{1,S(2)} = \omega_{1,S(1)} + 2n\omega_r, \quad (8a)$$

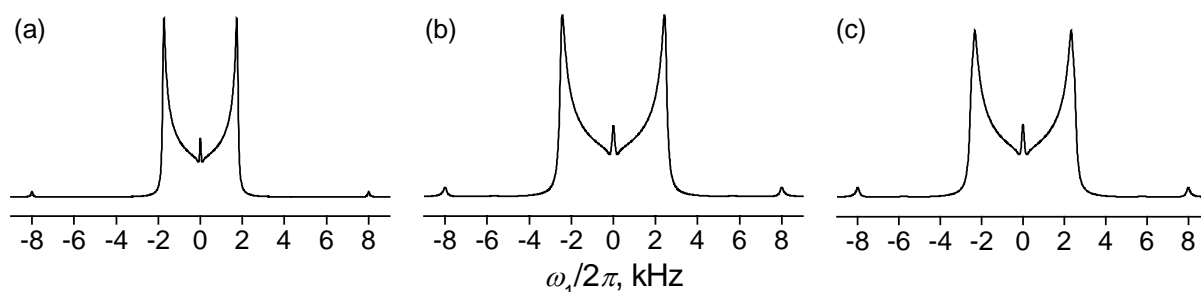
$$\omega_{1,I(2)} = \omega_{1,I(1)}. \quad (8b)$$

Hence, the rf field magnitudes in successive pulses in the  $S$  channel are alternated between the levels  $\gamma_C B_{1,C}^{(\pm)} = \gamma_H B_{1,H} \pm \omega_r$ . Alternatively, amplitude modulation can be performed in the  $I$  channel, or even in both channels.

As shown above, the lowest order average dipolar APM-CP Hamiltonian at ideal HH matching is equivalent to that in conventional CP. The advantage is, however, that the phase-alternation employed in APM-CP recoupling results in suppression of the effects of rf mismatch, frequency offsets and chemical shift interactions.<sup>1,3</sup>

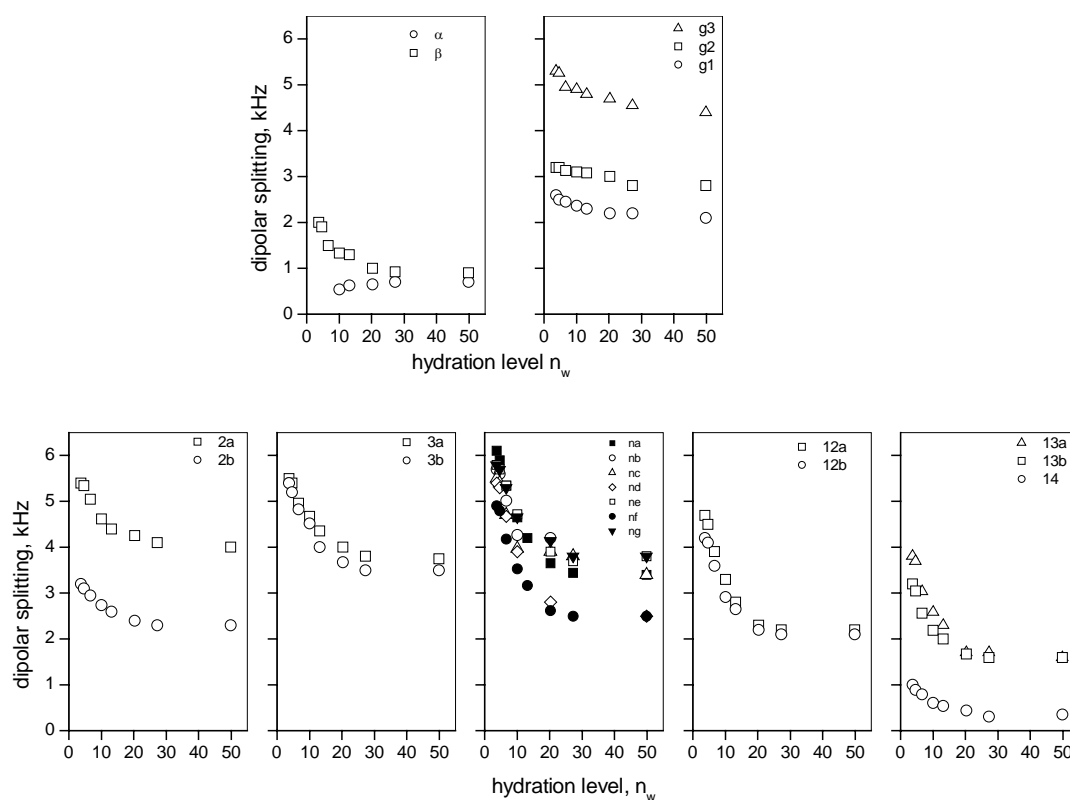
## B. NUMERICAL SIMULATIONS

A simulated APM-CP dipolar powder pattern of an isolated  $^1\text{H}$ - $^{13}\text{C}$  spin pair with a dipolar coupling of  $d_{\text{CH}}/2\pi = 5$  kHz is shown in Fig. S1a. The calculation assumes an rf field of  $\gamma_H B_{1,H}/2\pi = 80$  kHz and a spinning frequency of  $\omega_r/2\pi = 8$  kHz. The splitting between the two singularities is given by  $2\pi \times \Delta\nu_{\text{CH}} = (1/2)^{1/2} d_{\text{CH}} \approx 0.707 d_{\text{CH}}$ . The dipolar powder spectrum for a  $\text{CH}_2$  group has in general a rather complex shape, and is sensitive to the H-C-H bond angle.<sup>1,4</sup> For molecules undergoing fast axial motions, the effective bond angle becomes  $0^\circ$  (or  $180^\circ$ ). In this case, the  $^{13}\text{C}$  spin dynamics is analogous to that of a single CH spin pair with an effective dipolar coupling of  $d_{\text{CH}_2}^{\text{eff}} = \sqrt{d_{\text{CH}(1)}^2 + d_{\text{CH}(2)}^2}$ , where  $d_{\text{CH}(1)}$  and  $d_{\text{CH}(2)}$  are the motionally averaged heteronuclear interactions in the  $\text{CH}_2$  group. An important case often met in practice is when the residual dipolar couplings are equal, i.e., when  $d_{\text{CH}(1)} = d_{\text{CH}(2)} \equiv d_{\text{CH}_2}$  holds. In this situation, the spectral splitting becomes  $2\pi \times \Delta\nu_{\text{CH}_2} = d_{\text{CH}_2}$ . The effect due to the  $^1\text{H}$  homonuclear dipolar coupling  $d_{\text{HH}}$  within the  $\text{CH}_2$  group is minor at spinning frequencies  $\omega_r > d_{\text{HH}}$ . Simulated spectra of a rapidly rotating  $\text{CH}_2$  methylene group with equivalent residual dipolar couplings of 5 kHz excluding and including a  $^1\text{H}$ - $^1\text{H}$  dipolar interaction of  $d_{\text{HH}}/2\pi = 6$  kHz are shown, respectively, in Figs. S1b and S1c.



**Figure S1.** Simulated dipolar powder patterns using APM-CP recoupling of: (a) a C-H spin pair ( $d_{\text{CH}}/2\pi = 5$  kHz), and of axially rotating  $\text{CH}_2$  methylene groups ( $d_{\text{CH}(1)}/2\pi = d_{\text{CH}(2)}/2\pi = 5$  kHz) excluding (b) and including (c) the  $^1\text{H}$  homonuclear dipolar interaction ( $d_{\text{HH}}/2\pi = 6$  kHz). The exponential line broadening is 100 Hz in (a), and 200 Hz in (b) and (c). The simulations were performed using the SIMPSON software.<sup>5</sup>

### C. DIPOLAR SPLITTINGS AS A FUNCTION OF THE HYDRATION LEVEL



**Figure S2.** Dipolar splittings as a function of the hydration level in DMPC at 41 °C.

### D. REFERENCES

- (1) S. V. Dvinskikh, H. Zimmermann, A. Maliniak and D. Sandström, *J. Chem. Phys.* 2005, **122**, 044512.
- (2) M. H. Levitt, in *Encyclopedia of Nuclear Magnetic Resonance*, ed. D. M. Grant and R. K. Harris, Wiley, Chichester, 2002, p. 165.
- (3) M. Bjerring and N. C. Nielsen, *Chem. Phys. Lett.* 2003, **382**, 671.
- (4) S. V. Dvinskikh, H. Zimmermann, A. Maliniak and D. Sandström, *J. Magn. Reson.* 2003, **164**, 165.
- (5) M. Bak, J. T. Rasmussen and N. C. Nielsen, *J. Magn. Reson.* 2000, **147**, 296.