Supporting Information

Centerband-Only-Detection-of-Exchange ^{31}P nuclear magnetic resonance and phospholipid lateral diffusion: Theory, simulation and experiment

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Lamellarity of DMPC-AECHO LUVs

A sodium dithionite fluorescence quenching assay was used to determine the number of lipid bilayers per liposome. Because the lipid bilayer is impermeable to sodium dithionite, the reduction in NBD-PE fluorescence upon external addition of this quencher will correspond to the fraction of lipids on the outer leaflet of the lipid membrane which are accessible to the quencher. Approximately 1 mg of liposomes were suspended in 2 mL HEPES buffer and placed in a disposable sizing cuvette with a square aperture. The sample was allowed to equilibrate at 15 °C for 10 minutes prior to collecting measurements. The NBD-PE fluorescence of this sample was excited at 470 nm, and the resulting emission at 528 nm was monitored every second in a QuantaMaster PTI spectrofluorimeter (Photon Technology International, Lawrenceville, NJ). After an initial baseline was established, the

measurement was paused, and 20 µL of freshly made 1 M sodium dithionite in 1 M Tris (pH 10) was added and mixed before the measurement was resumed. After a new baseline was established, the measurement was paused again and 20 µL of 5% Triton (by weight) solution was added to disrupt completely the lipid bilayer integrity before measurement was resumed. The lamellarity of the LUV sample was tested before and after magic angle spinning at 6500 Hz for approximately 12 hours. Only 50% of the lipids were accessible to the sodium dithionite quencher in both cases, indicating that the bilayers maintained their integrity over the course of the NMR experiments while spinning at high temperature for long periods of time.

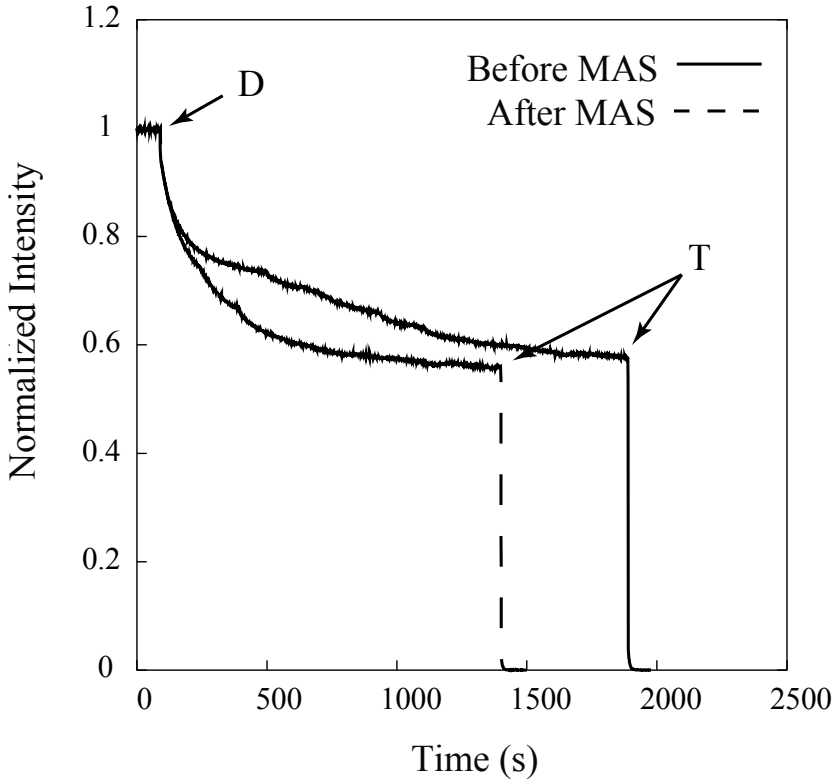


Figure S1. Sodium dithionite lamellarity assay performed on DMPC-AECHO LUVs before (solid line) and after (dashed line) MAS NMR. 20 µL of freshly made 1 M sodium dithionite in 1 M Tris (pH 10) was added at time D, and 20 µL of 5% Triton (by weight) solution was added at time T to disrupt the bilayer.

CODEX ^{31}P NMR Spectra in the Gel Phase

Fig. S2 (lower row) shows CODEX ^{31}P NMR spectra acquired at ν_r = 6500 Hz and M=3 as a function of increasing mixing time for LUV composed of 95/5 (mol/mol) DMPC/AECHO in 10 mM HEPES buffer, but at 10 °C, i.e., below the main gel-to-liquid-crystalline phase transition temperature of 23 °C. Also shown in Fig. S2 (upper row) are control experiments in which the mixing time t_m was held constant while the duration of the z-filter (t_z) was varied. Similar to the results for the liquid crystalline phase in Fig. 7, there is essentially no signal loss due to T_1 relaxation over the relevant time period in the gel phase, as expected given that the spin-lattice relaxation time T_1 for ^{31}P in gel phase DMPC bilayers is on the order ~ 1 s, while the correlation time of motion for lateral diffusion is on the order < 10 ms.

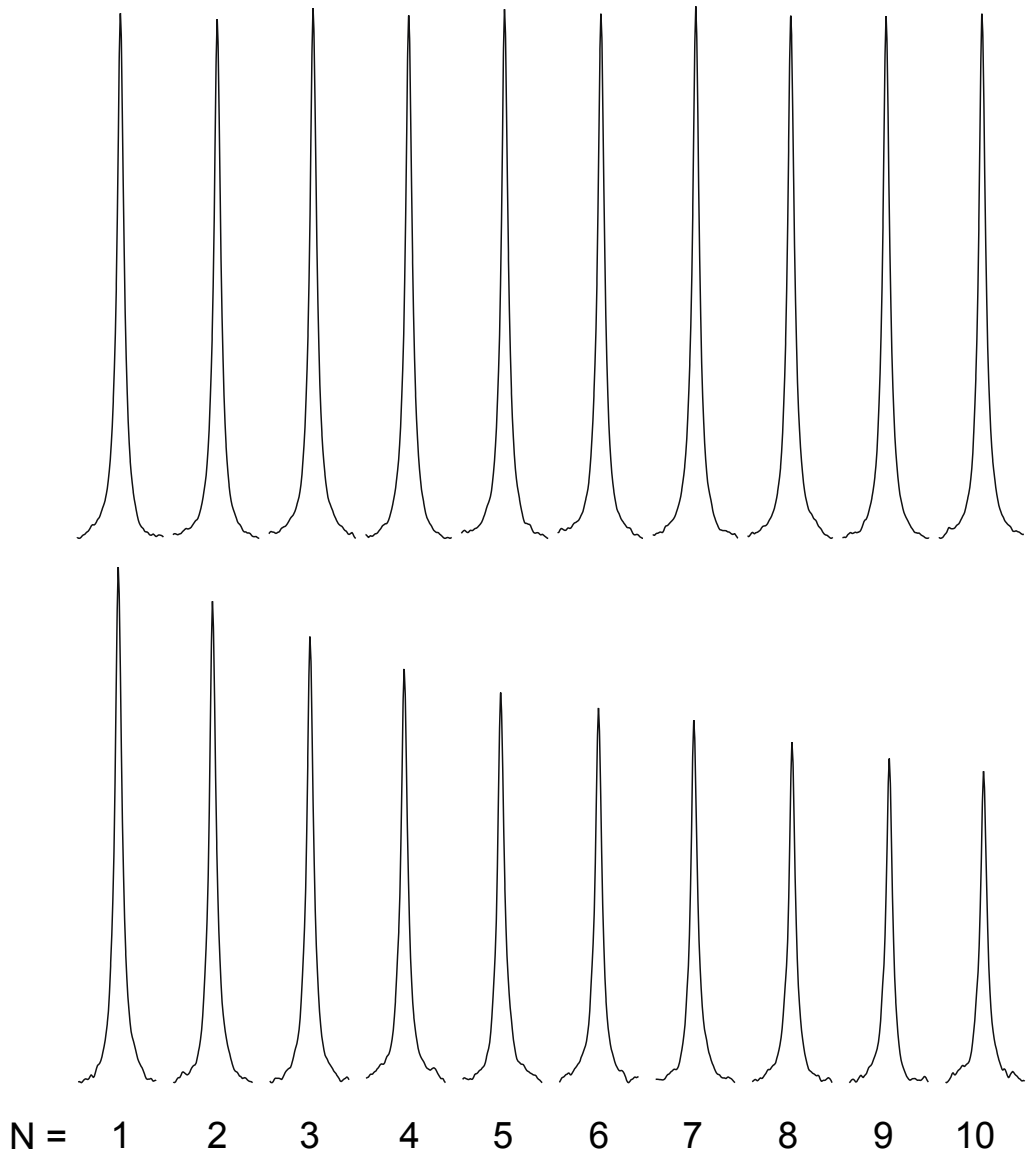


Figure S2. CODEX ^{31}P NMR spectra (M = 3) for LUV composed of DMPC/AECHO, 95/5, mol/mol, at 10 °C. The lower row shows the effects of increasing mixing time $t_m = Nt_r$, where $t_r = 154\ \mu s$ is the MAS rotor period at $\nu_r = 6500\ Hz$, for a constant value of the z-filter, $t_z = t_r$. The upper row shows the corresponding reference spectra in which the mixing time was held constant at $t_m = t_r$, while the duration of the z-filter was increased as $t_z = Nt_r$.

Effect of Deviations from Ideal Pulse Widths

Since phospholipid lateral diffusion produces a decrease in signal intensity with increasing mixing time in CODEX experiments, other potential sources of signal loss need to be minimized. Errors in the pulse widths will result in an incomplete recoupling of the CSA and concomitant signal loss, as shown in Fig. S3. Here, spectra were accumulated for the case $t_{mix} = t_r$, i.e., the shortest possible mixing time, corresponding to the initial spectrum of a CODEX mixing time array. Corresponding errors were deliberately introduced into the 90° and 180° pulse widths simultaneously, i.e., a 1 μs error in the 90° pulse width and a 2 μs error in the180° pulse width for the two cases M=1 and 3.

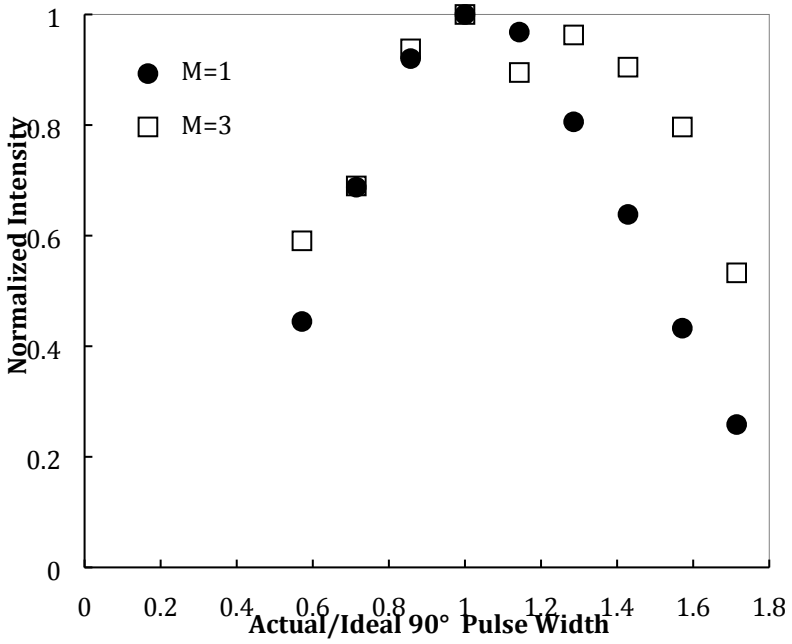
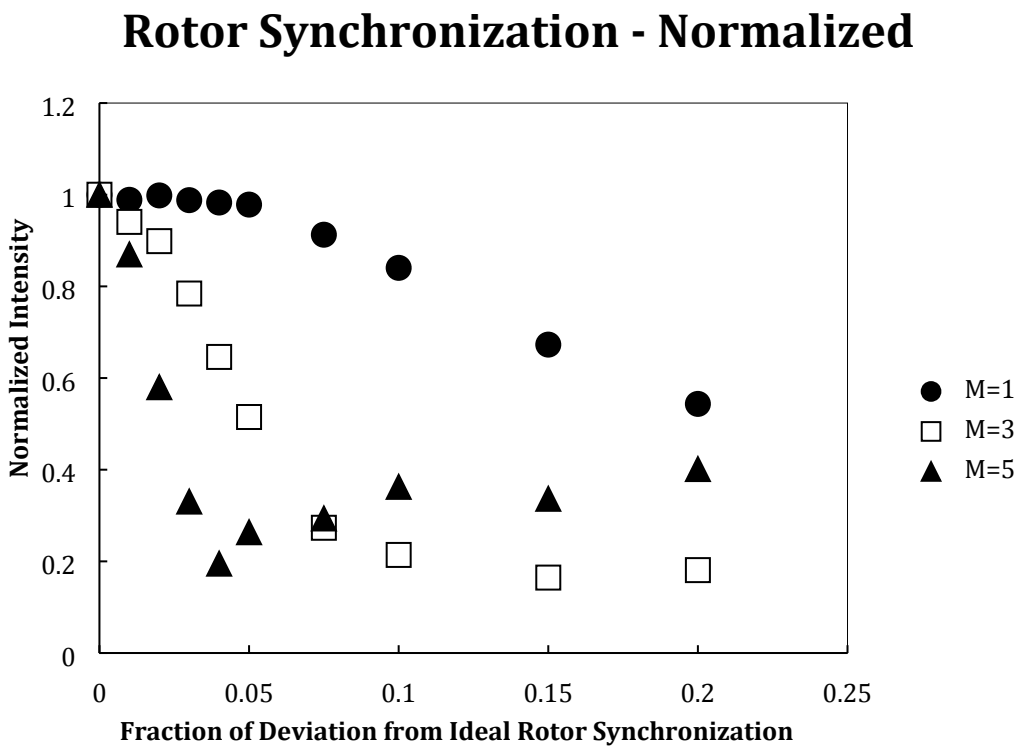


Figure S3. Normalized NMR intensity as a function of fractional deviations from the ideal 90° pulse width for M = 1 and M = 3, where M is the number of recoupling pulses. The CODEX ^{31}P NMR experiment was performed on LUV composed of DMPC/AECHO, 95/5, mol/mol, at 35 °C and $\nu_r = 6500\ Hz$. The shortest mixing time was used so that $t_m = t_r$, where $t_r = 154\ \mu s$.

Effect of Deviations from Ideal Rotor Synchronization

Rotor-synchronization of *rf* pulses is essential to CODEX recoupling of anisotropic interactions. In Fig. S4, the ideal pulse spacing of $t_r/2$ = 153.85/2 μs (for the case ν_r = 6500 Hz) was deliberately offset by a duration corresponding to some fraction of the ideal duration. Even slight de-synchronization profoundly attenuates the NMR signal, and increasingly so with increasing number of 180° recoupling pulses as the errors propagate. Either positive or negative offsets yielded essentially identical signal attenuations. We find that a convenient check of rotor synchronization is to inspect the intensity obtained with a high signal-to-noise ratio sample, like the multilmaellar vesicles in Fig. 8, at high M values as function of the pulse spacing.



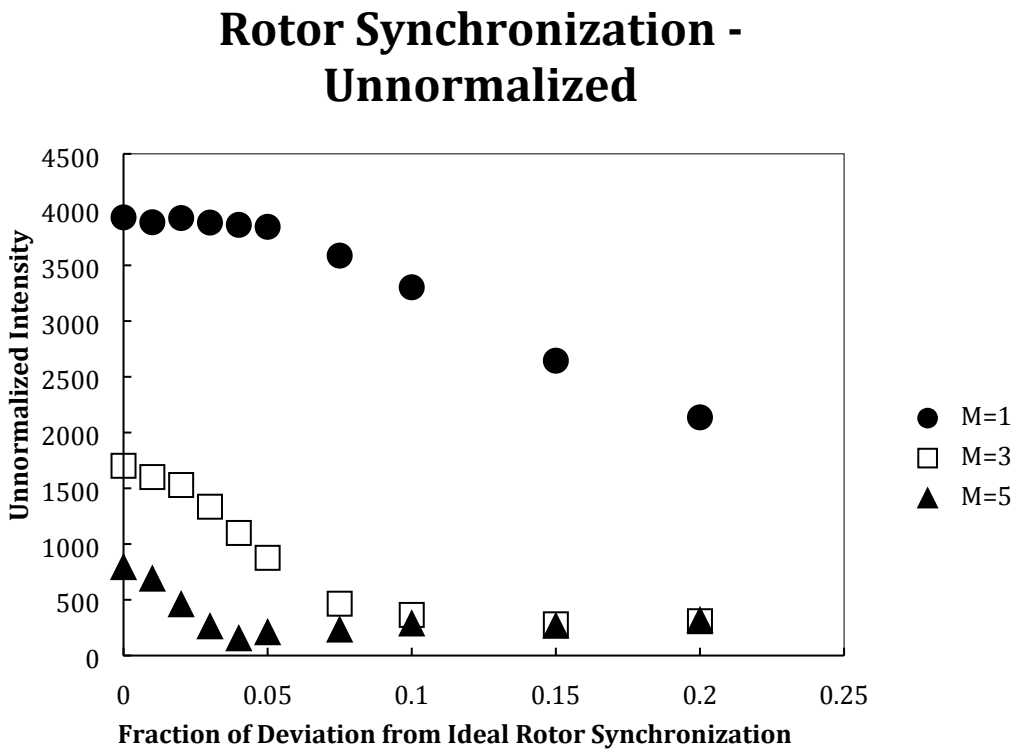
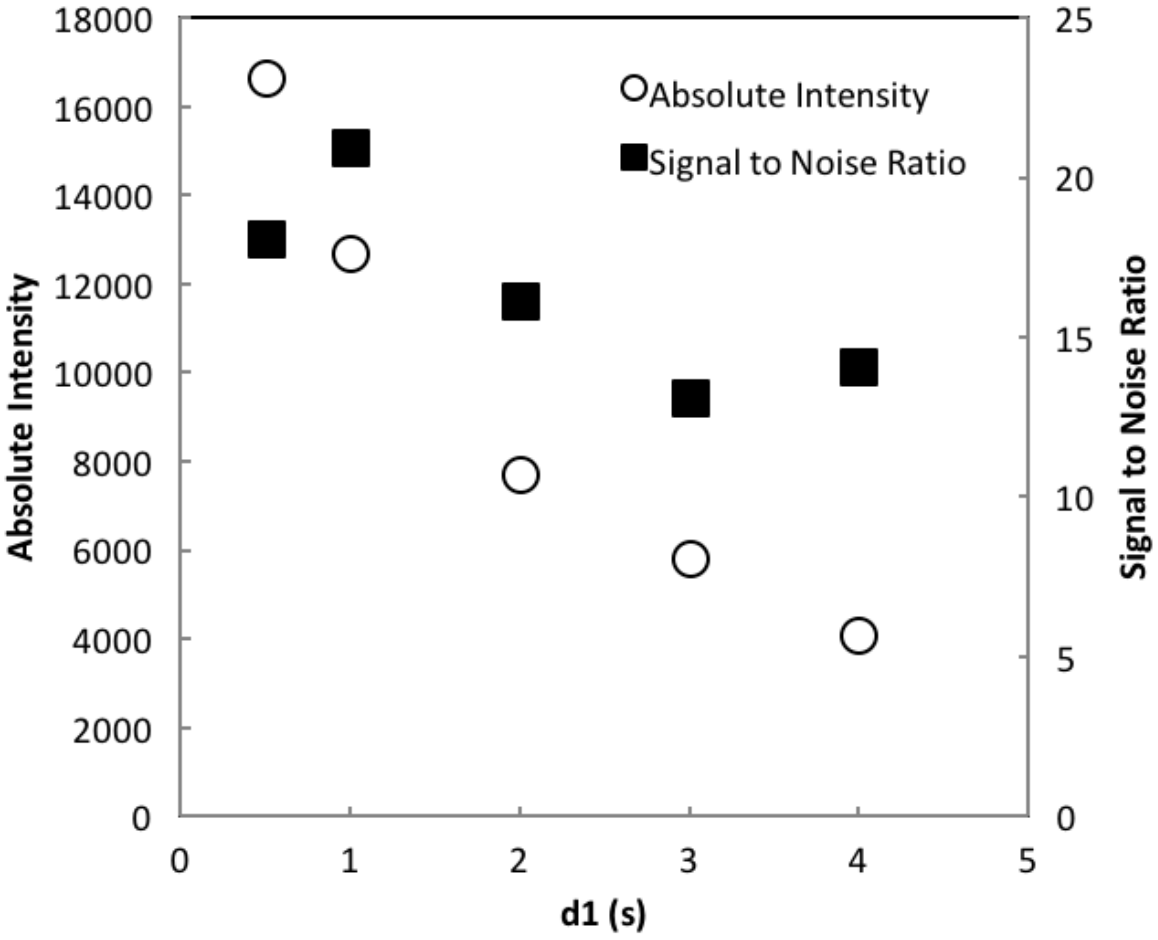


Figure S4. NMR signal intensity as a function of the fractional deviation of the pulse spacing from the ideal rotor synchronization. The CODEX ^{31}P NMR experiment was performed on LUV composed of DMPC/AECHO, 95/5, mol/mol, at 35 °C and $\nu_r = 6500\ Hz$. The shortest mixing time was used so that $t_m = t_r$, where $t_r = 153.85\ \mu s$. The pulse spacings were deliberately offset by some fraction of the ideal value ($t_r/2$) and the experiment was repeated for the cases M = 1, 3 and 5, where M is the number of 180° pulses during the recoupling period.

Effect of Deviations from Full Relaxation Conditions

Optimal lateral diffusion measurements involve maximizing signal-to-noise (SNR) for a given experimental duration, without deleteriously effecting the precision of the measurement. Optimal SNR for a given experimental duration can be obtained by use of a recycle delay (d1) shorter than the generally accepted value of $5xT_1$ necessary to achieve full relaxation between scans. At a T_1 of approximately 700 ms for our LUV at 35 °C, full relaxation requires a recycle delay of 3.5 s to allow for full relaxation. Fig. S5 shows the

^{31}P CODEX SNR obtained with LUV at 35 °C versus the recycle delay for constant experimental duration, i.e. the number of scans was adjusted inversely with the recycle delay. Decreasing the duration of the recycle delay from 4 s to 1 s produced a roughly 50% enhancement of SNR. From this plot, it is evident that decreasing the length of d1 from 4 s to 0.5 s greatly enhances signal intensity, with a d1 = 1 s spectrum having the optimum



SNR.

Figure S5. Signal to noise as a function of the recycle delay (d1) for a constant experiment duration, i.e., the number of scans was increased inversely with decreasing

recycle delay. The CODEX ^{31}P NMR experiment (M = 3) was performed on LUV composed of DMPC/AECHO, 95/5, mol/mol, at 35 °C and $\nu_r = 6500\ Hz$. The shortest mixing time was used so that $t_m = t_r$, where $t_r = 154\ \mu s$.

To examine whether shortening the recycle delay compromises the reliability of a lateral diffusion measurement, CODEX ^{31}P NMR diffusive decays were obtained using d1 = 2 s and 10 s for both M = 1 and M = 3, all other experimental parameters being kept constant. Fig. S6 compares the normalized signal decays, demonstrating that they are virtually superimposable. Thus, CODEX ^{31}P NMR lateral diffusion measurements can be performed in a much shorter period of time.

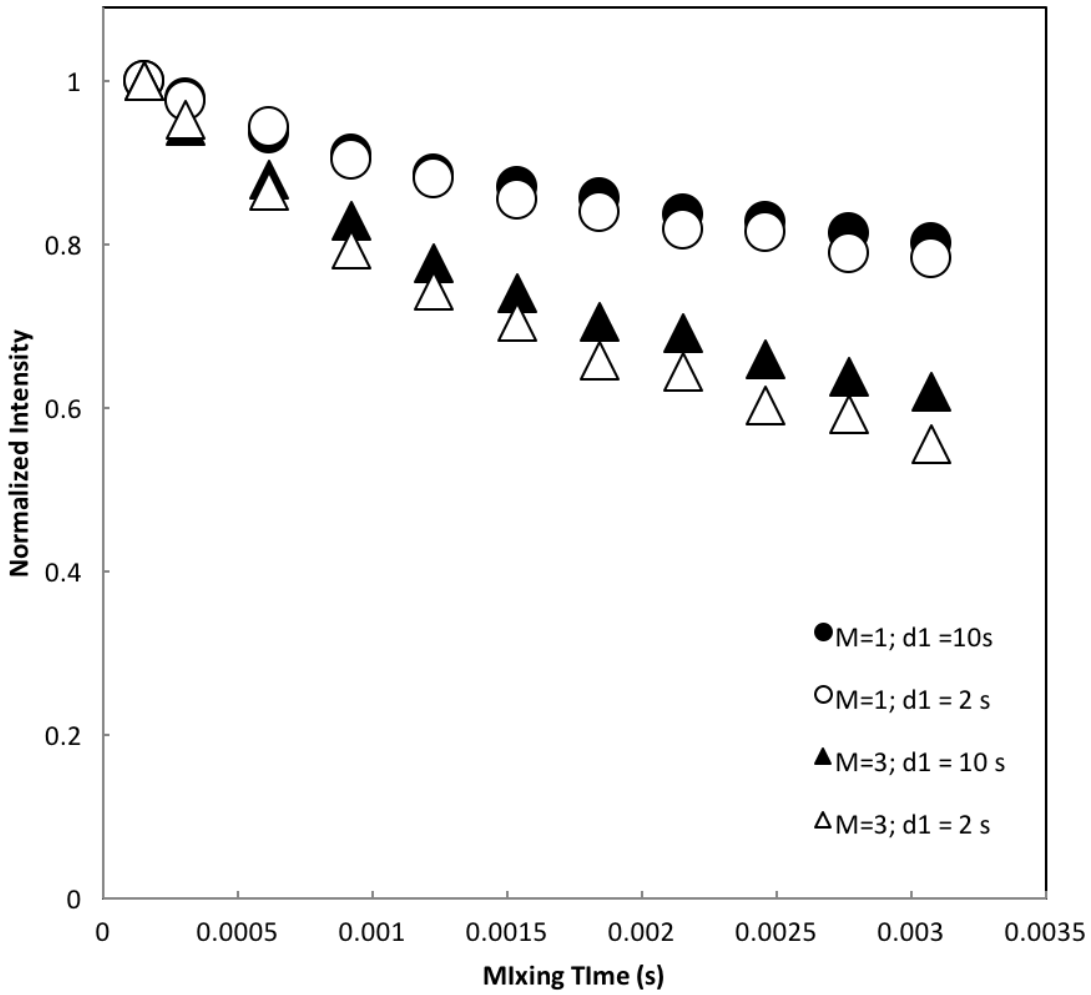


Figure S6. CODEX decays obtained with long (10 s) versus short (2 s) recycle delays for the M = 1 and M = 3 cases (where M denotes the number of 180° recoupling pulses). CODEX ^{31}P NMR spectra of MLV composed of DMPC/AECHO, 95/5, mol/mol, at 35 °C and $\nu_r = 6500\ Hz$, with increasing mixing times $t_m = Nt_r$, where $t_r = 154\ \mu s$ is the MAS rotor period, for a constant value of the z-filter, $t_z = t_r$.

Simulation of Slow MAS Spectra using SIMPSON

The powder average fitting approached detailed in this paper requires knowledge of the relevant CSA values. By spinning at a frequency below that of the relevant CSA values (ν_r = 2 kHz in this case), spinning sidebands will appear at frequencies separated by the spinning speed of 2 kHz and flanking the isotropic peak. These spectra can then be fit with a fitting software to extract the CSA values. Fig. S7 shows the experimental spectra in the solid blue lines at 35 °C and 10 °C, along with their SIMPSON simulation fits in the dotted red lines. For liquid-crystalline DMPC in LUV at 35°C a value of $3/2\delta = 43.07$ ppm was determined, while at 10°C the corresponding value was 61.26 ppm.

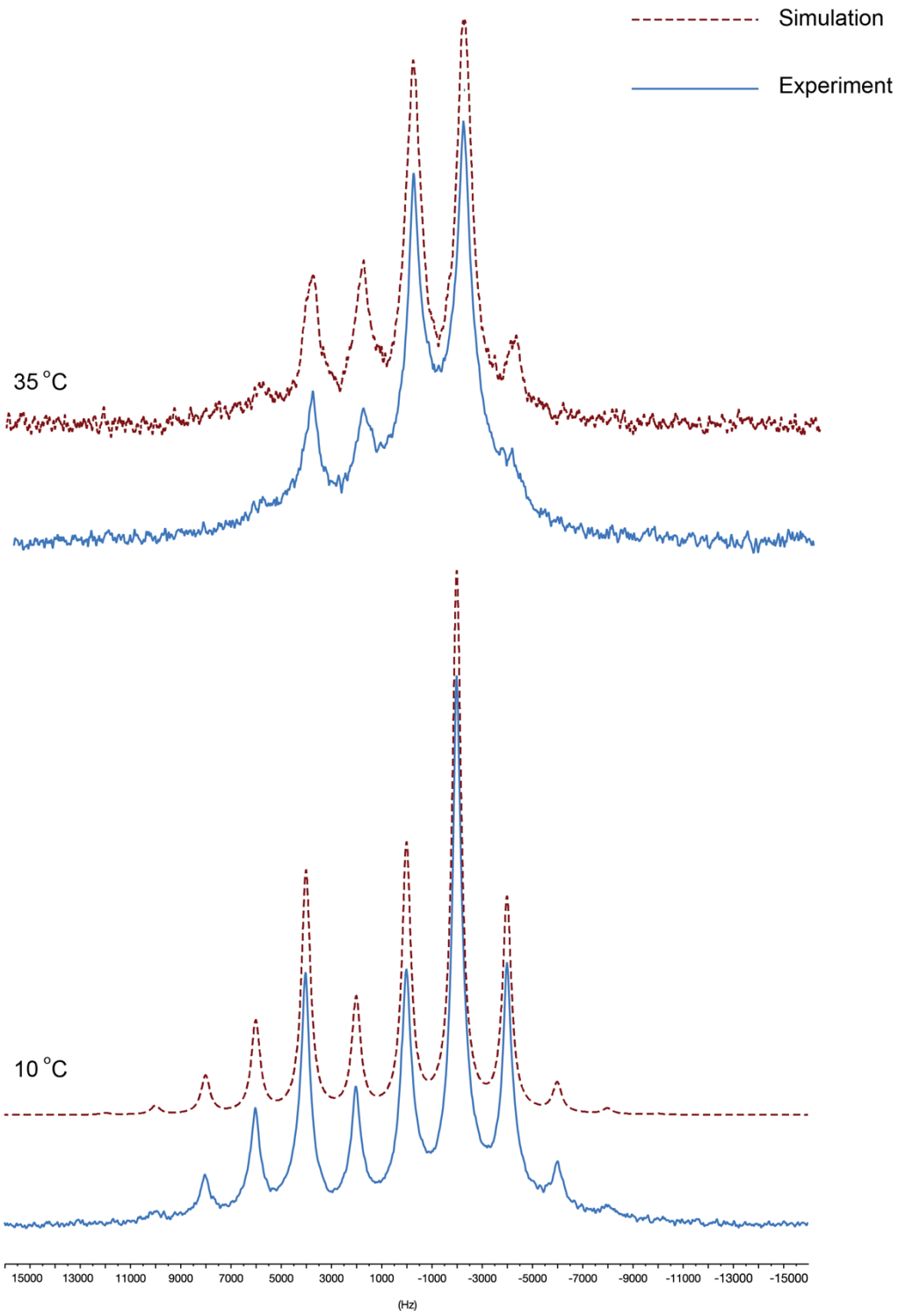


Figure S7. Slow magic angle spinning (ν_r = 2 kHz) ^{31}P NMR spectra of DMPC-AECHO LUVs at 35 °C and 10 °C and corresponding SIMPSON simulations. Experimental spectra are shown in the blue solid lines and SIMPSON simulations are shown in the red dashed lines. The SIMPSON simulations used a CSA of 43 ppm and 61 ppm, and line broadening of 740 and 420 Hz, at 35 °C and 10 °C, respectively, with asymmetry parameters near zero in both cases.