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Sensitivity Enhancement in Solid-State Separated Local Field NMR Experiments by the Use of Adiabatic Cross-Polarization

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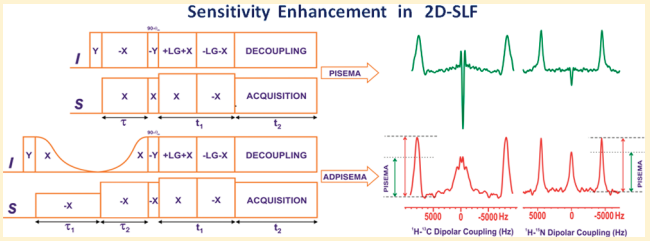
Supporting Information

ABSTRACT: Measurement of dipolar couplings using separated local field (SLF) NMR experiment is a powerful tool for structural and dynamics studies of oriented molecules such as liquid crystals and membrane proteins in aligned lipid bilayers. Enhancing the sensitivity of such SLF techniques is of significant importance in present-day solid-state NMR methodology. The present study considers the use of adiabatic cross-polarization for this purpose, which is applied for the first time to one of the well-known SLF techniques, namely, polarization inversion spin exchange at the magic angle (PISEMA). The experiments have been carried out on a single crystal of a model peptide, and a dramatic enhancement in signal-to-noise up to 90% has been demonstrated.



SECTION: Kinetics, Spectroscopy

Dipolar couplings obtained using solid-state NMR methods from static oriented samples have proved to be extremely useful for elucidating protein structures at atomic resolution, studying interactions of peptides and proteins with membranes, deriving the structure and topology of membrane proteins in lipid bilayers, and studying the dynamics and order in liquid-crystalline materials.[1−10] The commonly employed strategy for measuring dipolar couplings in these cases is to use the separated local field (SLF) technique, which provides site-specific information, as a two-dimensional plot between chemical shifts of nuclei such as ^{15}N or ^{13}C and the heteronuclear dipolar coupling of the nuclei to the neighboring protons.[11,12] Such techniques have been extensively used, for example, for structural studies of proteins embedded in biological membranes that are oriented either magnetically or by mechanical means.[2] However, NMR in general and solid-state NMR in particular suffer from problems of sensitivity, and this becomes an important issue when the available sample quantity is limited or preparation of a sufficient amount of isotopically labeled compound is difficult. Hence, techniques for enhancing sensitivity are being continuously explored, and some of the recent advances in this direction are the use of dynamic nuclear polarization[13,14] and the use of low-temperature MAS technology.[15] We present here an approach that can be used for enhancing the sensitivity of some of the SLF methodologies and report the first use of cross-polarization via the dipolar bath for such a purpose.[16−18] We apply this technique to one of the well-known SLF methodologies, namely, polarization inversion spin exchange at the magic angle (PISEMA),[19,20] and demonstrate the resulting significant increase in the signal-to-noise.

PISEMA is based on the transient oscillations observed during cross-polarization[21] and has the advantage of a relatively large scaling factor and a small line width.[22] PISEMA, however, has a few shortcomings such as the sensitivity of the measured dipolar couplings to positioning of the proton carrier frequency and sample heating effects due to use of long rf pulses. These problems have been addressed over a period of time, and several solutions have been suggested.[23−28] However, the problem of sensitivity of the experiment has attracted attention only recently, and some solutions have been proposed.[29,30] In this Letter, we consider the initial preparation period of the experiment, namely, polarization inversion (PI), as a possible means of increasing the sensitivity of the experiment. We propose the use of cross-polarization via the dipolar bath by the use of adiabatic demagnetization in the rotating frame (ADRF-CP),[18] to replace Hartmann−Hahn cross-polarization (HHCP),[18,31] originally used in PISEMA, and show that such a modification can enhance the signal-to-noise by as much as 90%.

It has been known for a long time that adiabatic or "total" cross-polarization provides the highest sensitivity enhancement in the solid state.[32−34] In contrast to HHCP (Figure 1a), where energy is conserved, in ADRF-CP (Figure 1b), entropy is conserved, and this leads to a much higher polarization for the dilute spin. However, this technique has not been in use very much as it is found to be inefficient under magic angle spinning conditions. We suggest here exploiting the favorable feature of the technique, for static oriented samples and single crystals, in two-dimensional SLF experiments. In this Letter, we initially present one-dimensional ^{13}C and ^{15}N spectra of static single crystals of *N*-acetyl-DL-valine (NAV) recorded with both HHCP

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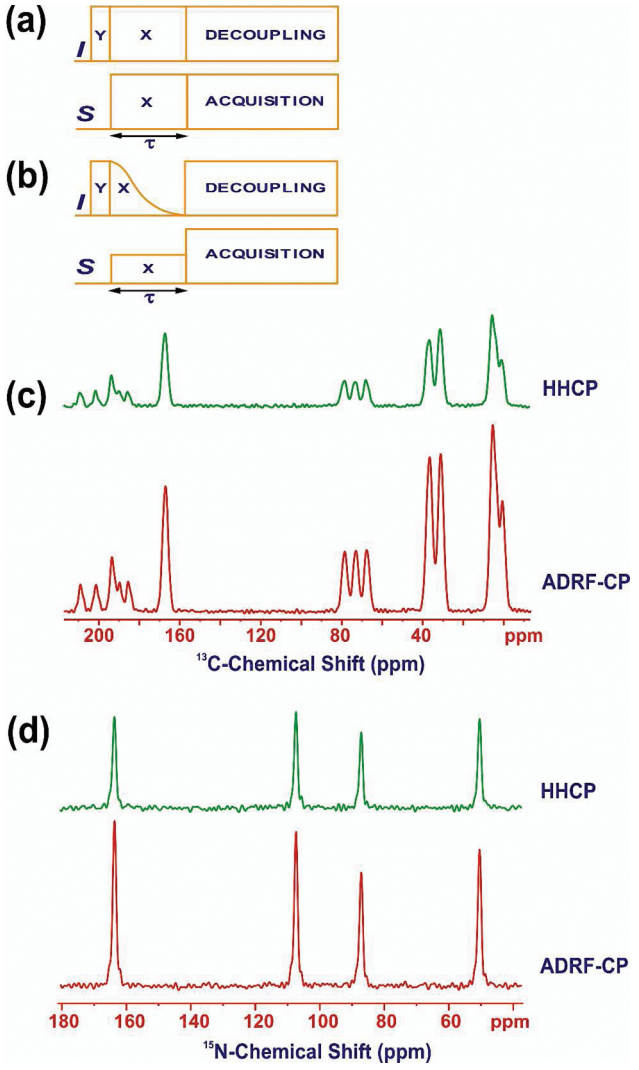

Figure 1. Pulse sequences for obtaining S spin spectrum with I spin decoupling using (a) HHCP and (b) ADRF-CP. The corresponding 1D ^{13}C and ^{15}N spectra of a single crystal of NAV for some arbitrary orientation are also shown. (c) ^{13}C spectra at natural abundance. For HHCP, a contact time of τ = 4 ms and 50 kHz rf power for the Hartmann−Hahn match condition were used. For ADRF-CP, a demagnetization time of τ = 20 ms and a proton rf field of 25.2 kHz (maximum value at the start of the ADRF sequence) and a carbon rf field of 24.2 kHz were used. A total number of 128 scans were acquired with a recycle time of 8 s. (d) ^{15}N spectra of ^{15}N-labeled NAV. For HHCP, a contact time of τ = 2 ms and 38.5 kHz rf power for the Hartmann−Hahn match condition were used. For ADRF-CP, a demagnetization time of τ = 20 ms and a proton rf field of 23.4 kHz (maximum value at the start of the ADRF sequence) and a nitrogen rf field of 17.8 kHz were used. A total number of 256 scans were acquired with a recycle time of 5 s.

and ADRF-CP and observe that ADRF-CP provides signal-to-noise ratios higher by factors ranging from 1.5 to 2.5 compared to those of HHCP. Subsequently, we incorporate ADRF-CP as the preparation period of PISEMA and demonstrate that with this modification, S/N increases by as much as 30−90%.

It was known earlier that the initial preparation period, namely, PI, of PISEMA has an important role in increasing the intensities of the dipolar cross-peaks.[19,35] This can be visualized by considering just the SEMA part of the experiment and starting with only the I

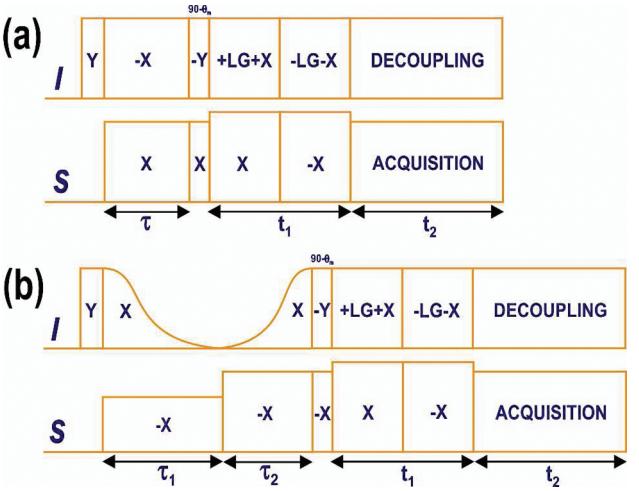

Figure 2. PISEMA pulse sequences with different schemes for polarization inversion: (a) conventional HHCP and (b) ADRF-CP.

spin magnetization, that is, without the τ period in Figure 2a.[36] In this case, the initial density matrix in the tilted rotating frame is given by $\sigma_0 = I_Z$. This can be divided into a double-quantum (DQ) density operator $I_Z^{DQ} = (1/2)(I_Z + S_Z)$ and a zero-quantum (ZQ) density operator $I_Z^{DQ} = (1/2)(I_Z - S_Z)$. Under Hartmann−Hahn match, with strong spin-lock rf, the ZQ density matrix evolves under the I−S dipolar coupling d to a term given by $(1/2)(I_Z - S_Z)\cos(d \sin \theta_m t)$, which upon Fourier transformation gives the dipolar cross-peaks in the 2D spectrum. Here, $\sin \theta_m$ is the scaling factor arising out of the proton magnetization spin-locked along the magic angle. The DQ density matrix essentially remains without evolution and gives rise to the axial-peak. As is evident from this discussion, the cross-peak intensity will increase if the ZQ component of the density matrix increases. I_Z^{ZQ} can be increased by polarizing the S spin and subsequently inverting this polarization. We may introduce a quantity k that represents the degree of polarization of the S spin and has values from 0 to 1. The relative ratio of cross-peak to axial-peak intensities can then be estimated by writing $I_Z^{ZQ} = [(1+k)/2](I_Z - S_Z)$ and $I_Z^{DQ} = [(1-k)/2](I_Z + S_Z)$. Thus, in the SEMA experiment discussed above, $k = 0$, and I_Z^{ZQ} and I_Z^{DQ} are of equal magnitude. In PISEMA, HHCP has been introduced during the τ period such that S spins are polarized by the I spin reservoir, and subsequently, the S spin polarization is reversed. In the ideal case, k can be 1, that is, the S spins are fully polarized, such that $I_Z^{ZQ} = (I_Z - S_Z)$ and $I_Z^{DQ} = 0$. In this case, the cross-peak intensity doubles, and the axial-peak vanishes. However under practical conditions, the enhancement of the cross-peak intensity depends on the degree of polarization of the S spin. It was observed earlier that the efficiency of HHCP in polarizing the S spin depends on the system and experimental conditions, and in some partially ordered liquid-crystalline systems, the use of a simple 90° pulse on the equilibrium magnetization of the S spin in the B_0 field provides PISEMA spectra better than those obtained with HHCP.[30,37] This is because with HHCP, the theoretical enhancement factor of γ_I/γ_S is not attained due to factors such as rf mismatch, averaging of dipolar couplings due to local motions, or the use of magic angle spinning and offset effects. There have been attempts that address these problems, and many modifications to the original HHCP have been suggested.[36,38,39] Here, we consider the ADRF-CP approach, which is fundamentally different from HHCP for creating S spin polarization. It has been

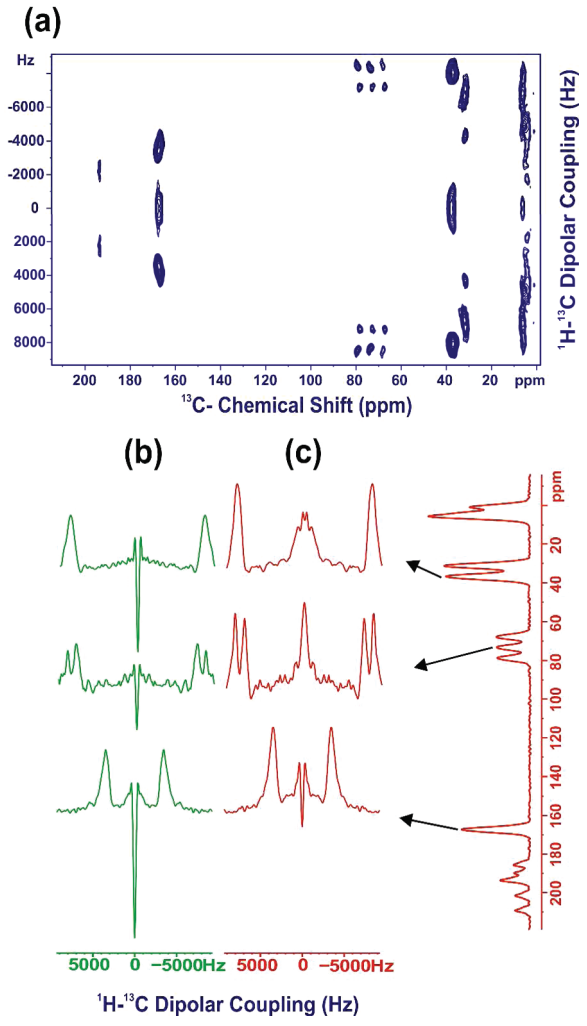

Figure 3. (a) Natural abundance ^{13}C 2D-ADPISEMA spectrum of a single crystal of NAV obtained using the pulse sequence shown in Figure 2b. Dipolar cross sections from the 2D spectrum are displayed below (c). Cross sections from the PISEMA experiment (2D spectrum not shown, pulse sequence shown as Figure 2a) have also been provided for comparison (b). The experimental parameters for the initial polarization period are the same as the ones given in the caption for Figure 1. For ADPISEMA, τ_1 is the same as τ in Figure 1b. The proton remagnetization period τ_2 was chosen to be 2 ms with a maximum rf field of 25.2 kHz for the proton channel and a spin-lock rf field of 50 kHz for the carbon channel. For the rest of the experiment, a ^{1}H rf field of 50 kHz was used. During acquisition SPINAL-64[41] decoupling was applied. Both 2D spectra were acquired for 96 t_1 points with 28 scans per t_1 increment with a recycle time of 8 s. 2D data sets of 4096 × 256 were double Fourier transformed in phase-sensitive mode and recorded with an exponential multiplication during t_2 and sine-bell apodization during t_1.

shown that the theoretical enhancement factor for ADRF-CP is $(\gamma_I/\gamma_S)(N_I/N_S)^{1/2}$, where N_I and N_S are the total number of I and S spins in the sample.[18] For I corresponding to protons and S corresponding to carbons or nitrogens, the theoretical enhancement that can be achieved with this approach is a very large factor compared to enhancement from HHCP.

Figure 1a and b shows, respectively, the HHCP and ADRF-CP pulse sequences. In ADRF-CP, spin-locked magnetization during the τ period is adiabatically transferred to the dipolar bath by reducing the proton rf field to zero. A rectangular rf pulse applied on the S spin-transfers polarization from the dipolar bath to the S spin Zeeman field in the rotating frame; rf can be applied on the S spin sequentially[18] or simultaneously[40] with respect to rf on the I spin. We found that simultaneous application of the rf fields both on I and S spins provided high S/N for the S spin. ^{13}C and ^{15}N spectra of a single crystal of NAV have been recorded using both HHCP and ADRF-CP using a Bruker AVANCE-III NMR spectrometer equipped with a solid-state double resonance probe with a horizontal solenoid coil for ^{13}C and a triple resonance 4 mm MAS probe under static condition for ^{15}N studies. The resonance frequencies for ^{1}H, ^{13}C, and ^{15}N were 500.18, 125.78, and 50.69 MHz, respectively. Figure 1c shows spectra of ^{13}C in natural abundance of a single crystal of NAV at an arbitrary orientation recorded using both HHCP and ADRF-CP pulse sequences with identical experimental conditions of relaxation delay, acquisition time, and proton decoupling power. Other relevant parameters have been provided in the figure captions. Similarly, Figure 1d shows ^{15}N HHCP and ADRF-CP spectra of a ^{15}N-labeled single-crystal sample of NAV. The choice of the parameters such as τ_1 and τ_2 and the rf power levels for the spectra shown in Figure 1 has been arrived at based on a systematic variation of the relevant parameters, the details of which are available in the Supporting Information. From Figure 1c and d, one may observe that ADRF-CP provides higher signal enhancements by factors ranging from 1.5 to 2.5 compared to HHCP.

Efficient polarization of the heteronucleus by ADRF-CP demonstrated above has been utilized for increasing the sensitivity of PISEMA (Figure 2a) by replacing HHCP with ADRF-CP for the polarization inversion part. The modified pulse sequence is shown in Figure 2b. The pulse sequence begins with the adiabatic demagnetization of I spins during τ_1, as in the case of the 1D experiment. Simultaneous rf applied on the S spin transfers polarization to the S spin. In the PISEMA experiment, I spin magnetization also needs to be recovered in order to create a density matrix term of the type $(I_Z - S_Z)$. Hence, a remagnetization pulse on the I spin is applied during τ_2. We observed that by this method, about 90% of the proton magnetization is recovered. During this period, the magnetization of carbon is preserved by the spin-lock rf. At the end of the τ_2 period, the experiment proceeds exactly like the PISEMA sequence. We call this modified sequence ADPISEMA.

^{13}C PISEMA and ADPISEMA spectra in natural abundance have been obtained for a single crystal of NAV for an arbitrary orientation. The ADPISEMA spectrum and a few typical cross sections from both PISEMA and ADPISEMA spectra are displayed in Figure 3. In the figure, it may be observed that peaks obtained from ADPISEMA show higher intensities by factors varying from 1.36 to 1.60, clearly demonstrating the sensitivity advantage of the ADPISEMA experiment. Similarly, ^{15}N spectra have been obtained from a ^{15}N-labeled single-crystalline sample of NAV. Both PISEMA and ADPISEMA experiments have been carried out, and the results are shown in Figure 4. In this case also, it is observed that ADPISEMA intensities are larger by 30−90%. We have listed in Table 1 representative sensitivity enhancement factors obtained for the ADPISEMA experiment over PISEMA for a few peaks. As explained elsewhere in this Letter, the maximum enhancement of intensity expected with the use of ADRF-CP is a factor of 2 or 100%, which would correspond to a case where HHCP is completely ineffective and ADRF-CP succeeds in fully polarizing the S spin. Other values would correspond to different degrees of polarization by the two schemes. The recovery of the proton magnetization in ADPISEMA during τ_2 will also have an

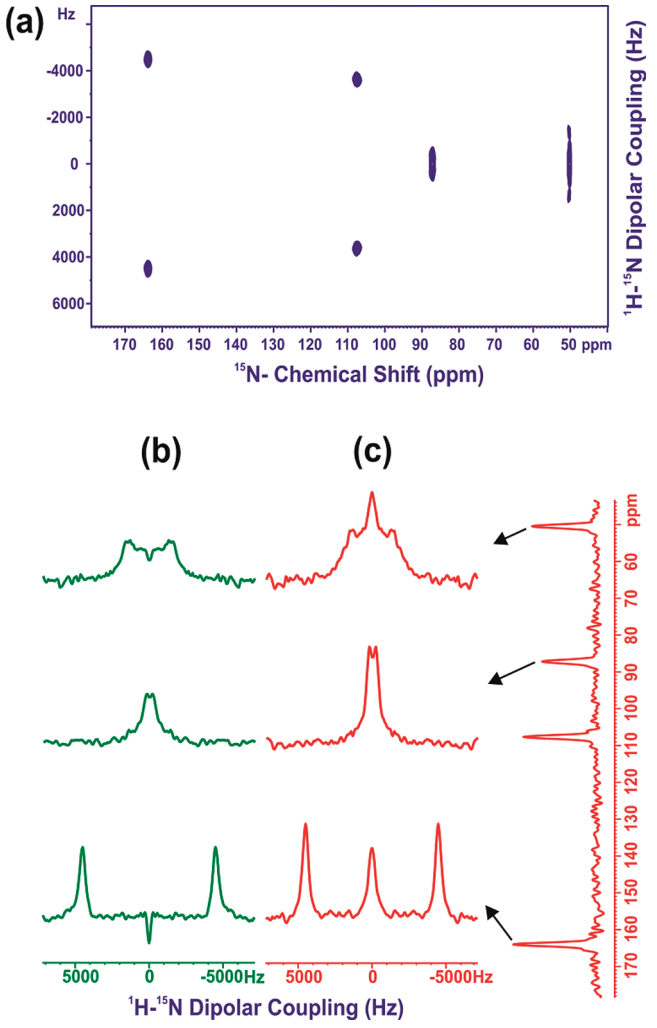

Figure 4. (a) ^{15}N 2D-ADPISEMA spectrum of a single crystal of ^{15}N-labeled NAV obtained using the pulse sequence shown in Figure 2b. Dipolar cross sections from the 2D spectrum are displayed below (c). Cross sections from the PISEMA experiment (2D spectrum not shown, pulse sequence shown as Figure 2a) have also been provided for comparison (b). The experimental parameters for the initial polarization period are the same as the ones given in the caption for Figure 1. For ADPISEMA, τ_1 is the same as τ in Figure 1b. The proton remagnetization period τ_2 was chosen to be 2 ms with a maximum rf field of 23.4 kHz for the proton channel and a spin-lock rf field of 38.5 kHz for the nitrogen channel. A ^{1}H rf field of 38.5 kHz was used for LG decoupling, and SPINAL-64 decoupling was applied during acquisition with a rf field of 62.5 kHz. Both 2D spectra were acquired for 100 t_1 points with 28 scans per t_1 increment with a recycle time of 5 s. 2D data sets of 4096 × 256 were double Fourier transformed in phase-sensitive mode and recorded with an exponential multiplication during t_2 and sine-bell apodization during t_1.

influence on deciding the enhancement factor. An enhancement factor greater than 1 indicates that ADRF-CP is more effective than HHCP, and this is found to be the case in the sample considered.

We also observed similar enhancement in intensities when ADPISEMA was applied to a few liquid-crystalline systems. The spectra shown here were recorded with a τ_1 = 20 ms, which is optimum for the NAV sample. It might however be useful to consider whether the technique will also be applicable to systems that have a short rotating frame relaxation time, $T_{1\rho}$. We have therefore carried out the ADPISEMA experiment for different

Table 1. Sensitivity Enhancement Factors for the ADPISEMA Experiment over the PISEMA Experiment for Some NAV Resonances

^{13}C chemical shift (ppm)	sensitivity enhancement	^{15}N chemical shift (ppm)	sensitivity enhancement
36.8	1.59	50.5	1.30
73.2	1.60	87.2	1.90
167.2	1.36	163.7	1.35

values of τ_1 starting from 5 ms for the ^{15}N case and have obtained values of signal enhancements relative to PISEMA. These results are shown in the Supporting Information (Figure S7). From these results, it is seen that the signal enhancements for ADPISEMA for τ_1 of 5 ms are about 85% of the value for τ_1 of 20 ms. In the case oriented biological samples, a range values for $T_{1\rho}$ from about 50 ms in the case of pure lipids[42] to about 5 ms in the case of proteins[43,44] have been reported. We therefore believe that the proposed modification to PISEMA will be useful in a variety of contexts.

It may be mentioned that for ADRF-CP, the rf powers used in both the proton and ^{13}C/^{15}N channels are low, comparable to the dipolar fields. On the other hand, in HHCP, a fairly large amount of rf power is required to keep the magnetization spin-locked. To avoid the use of rf power in the S channel during proton remagnetization time τ_2 (Figure 2b), it is possible to temporarily store the ^{13}C or ^{15}N magnetization in the Z direction with a pair of 90° pulses on the S channel. We observe that this modification to ADPISEMA also works equally well. We also notice with ADPISEMA the presence of a broad zero-frequency signal in some cases. As these peaks can interfere in the measurement of small dipolar couplings, they need to be eliminated. One possible approach would be to fine-tune the relative I and S magnetization to reduce the DQ magnetization and its contribution to the zero-frequency signal. This needs to be further investigated.

It may be mentioned that implementing the ADRF-CP sequence is straightforward and simple in modern spectrometers. It is possible to combine the present experiment with other sensitivity-enhancement techniques like SE-PISEMA,[29] in which case, a further gain in S/N will result. Further work incorporating ADRF-CP in other SLF methodologies like proton detected local field (PDLF)[45,46] spectroscopy and SAMPI-4[27] are in progress and will be reported elsewhere.

■ ASSOCIATED CONTENT

Supporting Information. Details of the procedure, adopted for optimization of the parameters used for obtaining the spectra shown in the main text, are described. Comparison of ADPISEMA intensities for different values of τ_1 is also presented. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Zeri, A. C.; Mesleh, M. F.; Nevzorov, A. A.; Opella, S. J. Structure of the Coat Protein in Fd Filamentous Bacteriophage Particles Determined by Solid-State NMR Spectroscopy. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6458–6463.

(2) Opella, S. J.; Marassi, F. M. Structure Determination of Membrane Proteins by NMR Spectroscopy. *Chem. Rev.* **2004**, *104*, 3587–3606.

(3) Dvinskikh, S. V.; Durr, U. H.; Yamamoto, K.; Ramamoorthy, A. High-Resolution 2D NMR Spectroscopy of Bicelles To Measure the Membrane Interaction of Ligands. *J. Am. Chem. Soc.* **2007**, *129*, 794–802.

(4) Page, R. C.; Li, C.; Hu, J.; Gao, F. P.; Cross, T. A. Lipid Bilayers: an Essential Environment for the Understanding of Membrane Proteins. *Magn. Reson. Chem.* **2007**, *45*, S2–S11.

(5) Traaseth, N. J.; Ha, K. N.; Verardi, R.; Shi, L.; Buffy, J. J.; Masterson, L. R.; Veglia, G. Structural and Dynamic Basis of Phospholamban and Sarcolipin Inhibition of Ca^{2+}-ATPase. *Biochemistry* **2008**, *47*, 3–13.

(6) Traaseth, N. J.; Verardi, R.; Torgersen, K. D.; Karim, C. B.; Thomas, D. D.; Veglia, G. Spectroscopic Validation of the Pentameric Structure of Phospholamban. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 14676–14681.

(7) Sinha, N.; Ramanathan, K. V.; Berdague, P.; Judeinstein, P.; Bayle, J. P. Ordering of a Lateral Crown Ether and Terminal Short POE Chains in Some Symmetrical Nematogens by ^{13}C NMR. *Liq. Cryst.* **2002**, *29*, 449–457.

(8) Xu, J.; Fodor-Csorba, K.; Dong, R. Y. Orientational Ordering of a Bent-Core Mesogen by Two-Dimensional ^{13}C NMR Spectroscopy. *J. Phys. Chem. A* **2005**, *109*, 1998–2005.

(9) Dvinskikh, S. V.; Yamamoto, K.; Scanu, D.; Deschenaux, R.; Ramamoorthy, A. High-Resolution Characterization of Liquid-Crystalline [60]Fullerenes Using Solid-State Nuclear Magnetic Resonance Spectroscopy. *J. Phys. Chem. B* **2008**, *112*, 12347–12353.

(10) Ramanathan, K. V.; Sinha, N. in Current Developments in Solid State NMR Spectroscopy; Muller, N., Madhu, P. K. Eds.; Springer-Verlag: Wien, Austria, 2003, Vol. III.

(11) Hester, R. K.; Ackerman, J. L.; Cross, V. R.; Waugh, J. S. Resolved Dipolar Coupling Spectra of Dilute Nuclear Spins in Solids. *Phys. Rev. Lett.* **1975**, *34*, 993–995.

(12) Waugh, J. S. Uncoupling of Local Field Spectra in Nuclear Magnetic Resonance: Determination of Atomic Positions in Solids. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1394–1397.

(13) Maly, T.; Debelouchina, G. T.; Bajaj, V. S.; Hu, K.-N.; Joo, C. G.; Mak-Jurkauskas, M. L.; Sirigiri, J. R.; van der Wel, P. C. A.; Herzfeld, J.; Temkin, R. J.; Griffin, R. G. Dynamic Polarization at High Magnetic Fields. *J. Chem. Phys.* **2008**, *128*, 052211/1–052211/19.

(14) Lesage, A.; Lelli, M.; Gajan, D.; Caporini, M. A.; Vitzthum, V.; Mieville, P.; Alauzun, J.; Roussey, A.; Thieuleux, C.; Medhi, A.; Bodenhausen, G.; Coperet, C.; Emsley, L. Surface Enhanced NMR Spectroscopy by Dynamic Nuclear Polarization. *J. Am. Chem. Soc.* **2010**, *132*, 15459–15461.

(15) Thurber, K. R.; Tycko, R. Biomolecular Solid State NMR with Magic Angle Spinning at 25 K. *J. Magn. Reson.* **2008**, *195*, 179–186.

(16) Slichter, C.; Holton, W. C. Adiabatic Demagnetization in a Rotating Reference System. *Phys. Rev.* **1961**, *122*, 1701–1708.

(17) Anderson, A.; Hartmann, S. R. Nuclear Magnetic Resonance in the Demagnetized State. *Phys. Rev.* **1962**, *128*, 2023–2041.

(18) Pines, A.; Gibby, M. G.; Waugh, J. S. Proton enhanced NMR of Dilute Spins in Solids. *J. Chem. Phys.* **1973**, *59*, 569–590.

(19) Wu, C. H.; Ramamoorthy, A.; Opella, S. J. High-Resolution Heteronuclear Dipolar Solid-State NMR Spectroscopy. *J. Magn. Reson. A* **1994**, *109*, 270–272.

(20) Ramamoorthy, A.; Wei, Y.; Lee, D. K. PISEMA Solid State NMR Spectroscopy. *Ann. Rev. NMR Spectrosc.* **2004**, *52*, 1–52.

(21) Muller, L.; Kumar, A.; Baumann, T.; Ernst, R. R. Transient Oscillations in NMR Cross-Polarization Experiments in Solids. *Phys. Rev. Lett.* **1974**, *32*, 1402–1406.

(22) Gan, Z. Spin Dynamics of Polarization Inversion Spin Exchange at the Magic Angle in Multiple Spin Systems. *J. Magn. Reson.* **2000**, *143*, 136–143.

(23) Nishimura, K.; Naito, A. Dramatic Reduction of the R.F. Power for Attenuation of Sample Heating in 2D-Separated Local Field Solid-State NMR Spectroscopy. *Chem. Phys. Lett.* **2005**, *402*, 245–250.

(24) Yamamoto, K.; Lee, D. K.; Ramamoorthy, A. Broadband-PISEMA Solid-State NMR Spectroscopy. *Chem. Phys. Lett.* **2005**, *407*, 289–293.

(25) Dvinskikh, S. V.; Sandstrom, D. Frequency Offset Refocused PISEMA-Type Sequences. *J. Magn. Reson.* **2005**, *175*, 163–69.

(26) Nishimura, K.; Naito, A. Remarkable Reduction of rf Power by ATANSEMA and DATANSEMA Separated Local Field in Solid-State NMR Spectroscopy. *Chem. Phys. Lett.* **2006**, *419*, 120–124.

(27) Nevzorov, A. A.; Opella, S. J. Selective Averaging for High-Resolution Solid-State NMR Spectroscopy of Aligned Samples. *J. Magn. Reson.* **2007**, *185*, 59–70.

(28) Jayanthi, S.; Ramanathan, K. V. 2_n-SEMA — A Robust Solid State Nuclear Magnetic Resonance Experiment for Measuring Heteronuclear Dipolar Couplings in Static Oriented Systems Using Effective Transverse Spin-Lock. *J. Chem. Phys.* **2010**, *132*, 134501/1–134501/9.

(29) Gopinath, T.; Veglia, G. Sensitivity Enhancement in Static Solid-State NMR Experiments via Single and Multiple-Quantum Dipolar Coherences. *J. Am. Chem. Soc.* **2009**, *131*, 5754–5756.

(30) Das, B. B.; Ajithkumar, T. G.; Ramanathan, K. V. Enhancing Cross-Peak Intensity in 2D-SLF Spectroscopy — The Role of Equilibrium Carbon Magnetization in Cross-Polarization Experiments. *Chem. Phys. Lett.* **2006**, *426*, 422–425.

(31) Hartmann, S. R.; Hahn, E. L. Nuclear Double Resonance in the Rotating Frame. *Phys. Rev.* **1962**, *128*, 2042–2053.

(32) Mehring, M. *Principles of High Resolution NMR in Solids*; Springer-Verlag: Berlin, Germany, 1983.

(33) Pines, A.; Shattuck, T. W. Carbon-13 Proton NMR Cross-Polarization Times in Solid Adamantane. *J. Chem. Phys.* **1974**, *61*, 1255–1256.

(34) Pines, A.; Chang, J. J.; Griffin, R. G. Carbon-13 Nuclear Magnetic Resonance in Solid Ammonium Tartrate. *J. Chem. Phys.* **1974**, *61*, 1021–1030.

(35) Sinha, N.; Ramanathan, K. V. Use of Polarization Inversion for Resolution of Small Dipolar Couplings in SLF-2D NMR Experiments-an Application to Liquid Crystals. *Chem. Phys. Lett.* **2000**, *332*, 125–130.

(36) Levitt, M. H.; Sutar, D.; Ernst, R. R. Spin Dynamics and Thermodynamics in Solid-State NMR Cross Polarization. *J. Chem. Phys.* **1986**, *84*, 4243–4255.

(37) Das, B. B.; Ajithkumar, T. G.; Sinha, N.; Opella, S. J.; Ramanathan, K. V. Cross- and Axial-Peak Intensities in 2D-SLF Experiments Based on Cross-Polarization-The Role of the Initial Density Matrix. *J. Magn. Reson.* **2007**, *185*, 308–317.

(38) Metz, G.; Wu, X.; Smith, S. O. Ramped-Amplitude Cross Polarization in Magic-Angle-Spinning NMR. *J. Magn. Reson.* **1994**, *A110*, 219–227.

(39) Fukuchi, M.; Ramamoorthy, A.; Takegoshi, K. Efficient Cross-Polarization Using a Composite 0° Pulse for NMR Studies on Static Solids. *J. Magn. Reson.* **2009**, *196*, 105–109.

(40) Lee, J. S.; Khitrin, A. K. Thermodynamics of Adiabatic Cross Polarization. *J. Chem. Phys.* **2008**, *128*, 114504/1–114504/7.

(41) Fung, B. M.; Khitrin, A. K.; Ermolaev, K. An Improved Broadband Decoupling Sequence for Liquid Crystals and Solids. *J. Magn. Reson.* **2000**, *142*, 97–101.

(42) Separovic, F.; Cornell, B.; Pace, R. Orientation Dependence of NMR Relaxation Time, $T_{1\rho}$, in Lipid Bilayers. *Chem. Phys. Lipids* **2000**, *107*, 159–167.

(43) Cady, S. D.; Goodman, C.; Tatko, C. D.; DeGrado, W. F.; Hong, M. Determining the Orientation of Uniaxially Rotating Membrane Proteins Using Unoriented Samples: A ^{2}H, ^{13}C, and ^{15}N Solid-State NMR Investigation of the Dynamics and Orientation of a Transmembrane Helical Bundle. *J. Am. Chem. Soc.* **2007**, *129*, 5719–5729.

(44) Kamihira, M.; Watts, A. Functionally Relevant Coupled Dynamic Profile of Bacteriorhodopsin and Lipids in Purple Membranes. *Biochemistry* **2006**, *45*, 4304–4313.

(45) Caldarelli, S; Hong, M; Emsley, L Pines, Measurement of Carbon−Proton Dipolar Couplings in Liquid Crystals by Local Dipolar Field NMR Spectroscopy. *J. Phys. Chem.* **1996**, *100*, 18696–18701.

(46) Caldarelli, S; Lesage, A.; Emsley, L. A. Long-Range Dipolar Couplings in Liquid Crystals Measured by Three-Dimensional NMR Spectroscopy. *J. Am. Chem. Soc.* **1996**, *118*, 12224–12225.