Supporting Information for the manuscript

Accurate Determination of Order Parameters from ^{1}H, ^{15}N dipolar couplings in MAS solid-state NMR experiments

by

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Effect of radiofrequency inhomogeneity

The most severe source of error in the determination of ^{1}H-^{15}N dipolar couplings originates from rf field inhomogeneities which are intrinsic to any particular probe. The magnetization S_N which is transferred from ^{1}H to ^{15}N during CP (assuming the ±1 Hartmann-Hahn matching condition) is calculated for a given CP contact time τ according to[1]

$$S_N \propto \frac{|b_{\pm 1}|^2}{|b_{\pm 1}|^2 + (\Delta - \omega_r)^2} \sin\left\{\frac{\tau}{2}\sqrt{|b_{\pm 1}|^2 + (\Delta - \omega_r)^2}\right\}$$

where the ω_r corresponds to the rotor spinning frequency, and $\Delta = \omega_{1H} - \omega_{1N}$ to the rf fields difference applied on the proton and nitrogen channel, respectively. $b_{\pm 1}$ is determined by the internuclear distance r_{NH} and the angle β between the principal axis of the ^{1}H-^{15}N dipolar tensor and the rotor axis

$$b_{\pm 1} = \frac{\sqrt{2}}{4} \frac{\gamma_H \gamma_N \hbar}{r_{NH}^3} \sin(2\beta)$$

$\hbar$ denotes Planck constant, the proton and nitrogen gyromagnetic ratios are given by γ_H and γ_N. A misadjustment of the CP condition due to rf inhomogeneity on either the ^{1}H channel, the ^{15}N channel or both, results in an apparently faster oscillations and yields an overestimation of the measured dipolar coupling. A ^{1}H-^{15}N dipolar coupling of 10 kHz corresponds to $b_{\pm 1} = 7071$ Hz for spin pairs with β=45. Already a rf field inhomogeneity on the order of 1 kHz increases significantly the apparent proton-nitrogen dipolar coupling (see Supporting Figure 1).
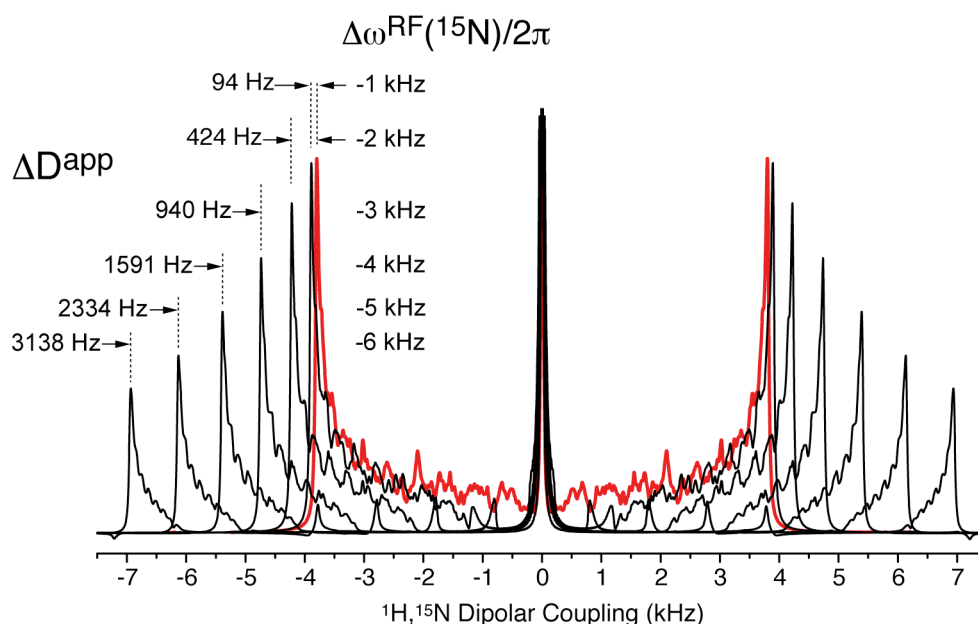
Supporting Figure 1. Effect of RF inhomogeneity on the appararent dipolar oscillation in a regular CP experiment. The spectra were simulated using the program SIMPSON.[2] In the ideal case, the ^{1}H RF and ^{15}N RF fields were set to 56 kHz and 36/76 kHz, respectively. For simulation of the effect of rotary resonance mismatch, the ^{15}N RF field was decreased from 36 kHz to 30 kHz in steps of 1 kHz. Typical values for RF inhomogeneity on a standard triple resonance probe are on the order of 10 %. The MAS rotation frequency was assumed to be 20 kHz. A mismatch of 6 kHz of the ^{15}N RF field results in almost a doubling of the apparent dipolar oscillation frequency.
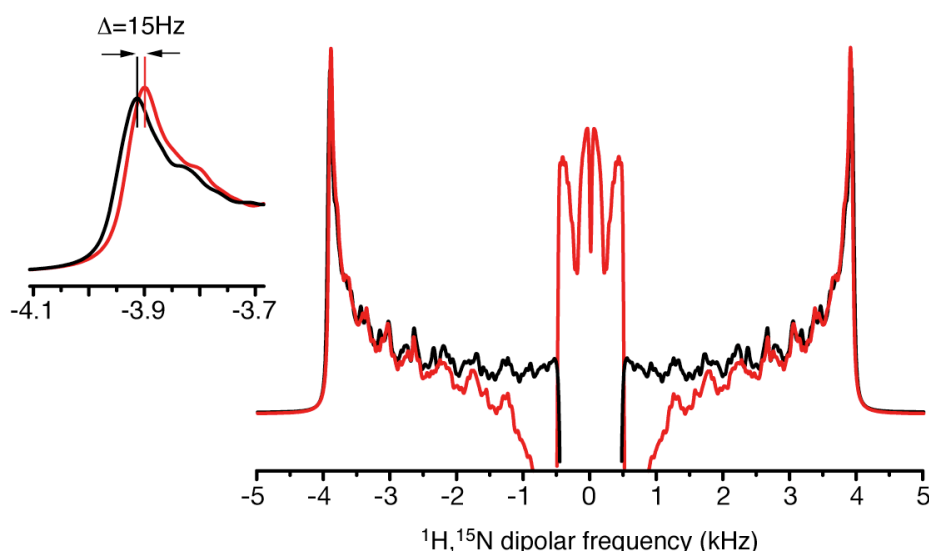

Supporting Figure 2. Simulation of ^{1}H,^{15}N dipolar oscillations in the Phase-Inverted CP experiment (CPPI) under RF inhomogeneity. The spectra were simulated using the program SIMPSON.[2] In the ideal case, the ^{1}H RF and ^{15}N RF fields were set to 56 kHz and 36/76 kHz, respectively. To simulate the effect of RF mismatch, the ^{15}N RF field was set to 30/70 kHz. The MAS rotation frequency was assumed to be 20 kHz. RF inhomogeneity induces a variation of the diplor oscillation in the order of 15 Hz.
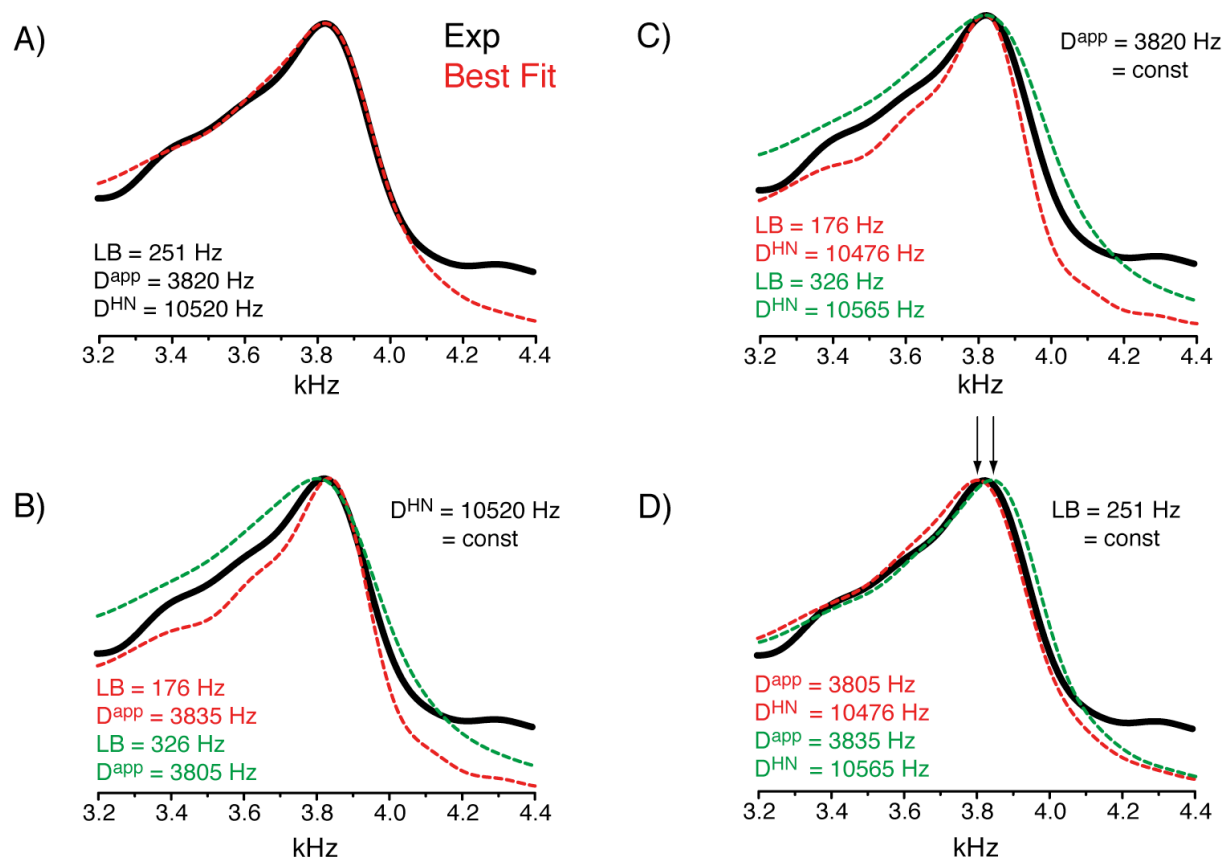
Supporting Figure 3. Estimation of the error in the determination of the $^1H,^{15}N$ dipolar couplings. In all panels, the right part of the doublet is drawn only. All panels display simulation and experimental data for the dipolar oscillation observed for residue K18. In all cases, simulated data are drawn as red or green dashed lines, whereas experimental data are represented by a solid line.

A) Experimental data and best fit. B) As the simulation program does not include a damping term to estimate the decay of magnetization, simulated data are apodized by multiplication with an exponentially decaying function.
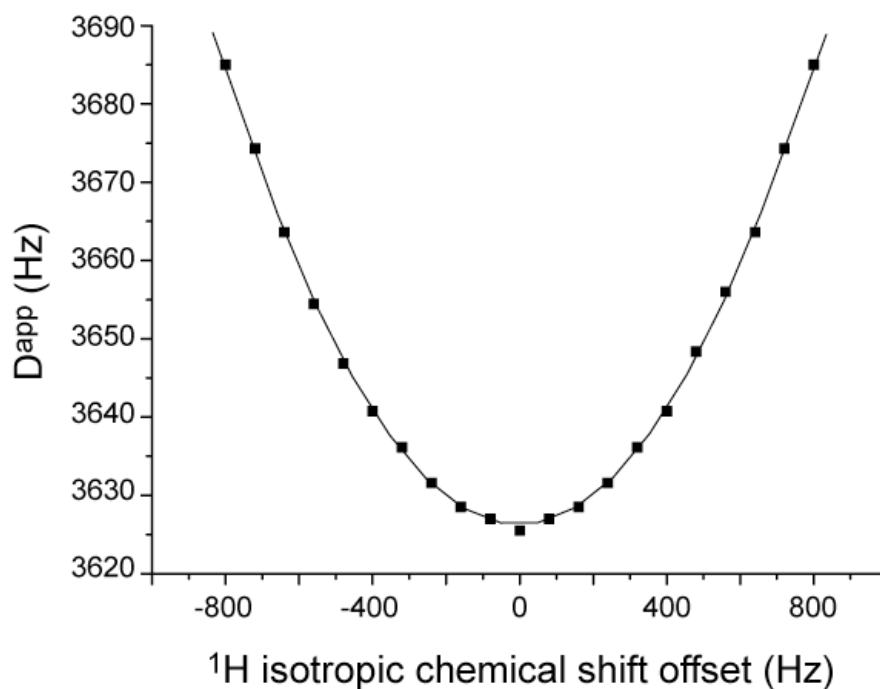
Supporting Figure 4. Dependence of the experimental dipolar splitting D^{app} in the CPPI experiment on the offset of the $^1H^N$ isotropic chemical shift of an individual amide proton. Dipolar couplings shown in Figures 3 and 4 of the manuscript are corrected individually for chemical shift offset. The simulation was carried out using the program SIMPSON.[2]
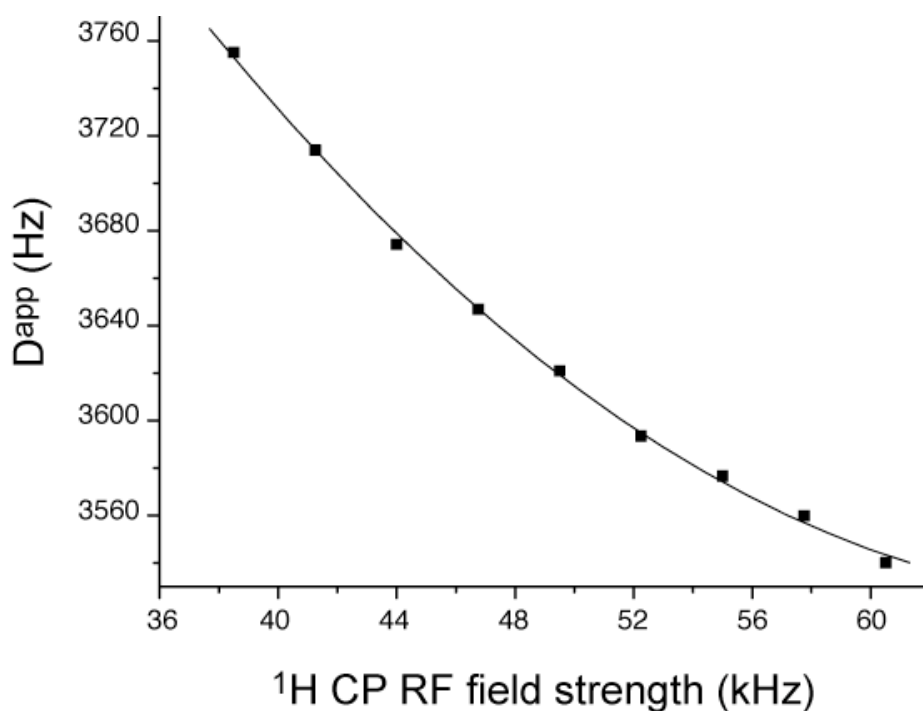
Supporting Figure 5. Dependence of the experimental dipolar splitting D^{app} on the ^1H RF field strength employed in the CPPI experiment. Dipolar couplings represented in Figures 3 and 4 were analyzed using the experimental ^1H RF field strengths values explicitly in the simulation.
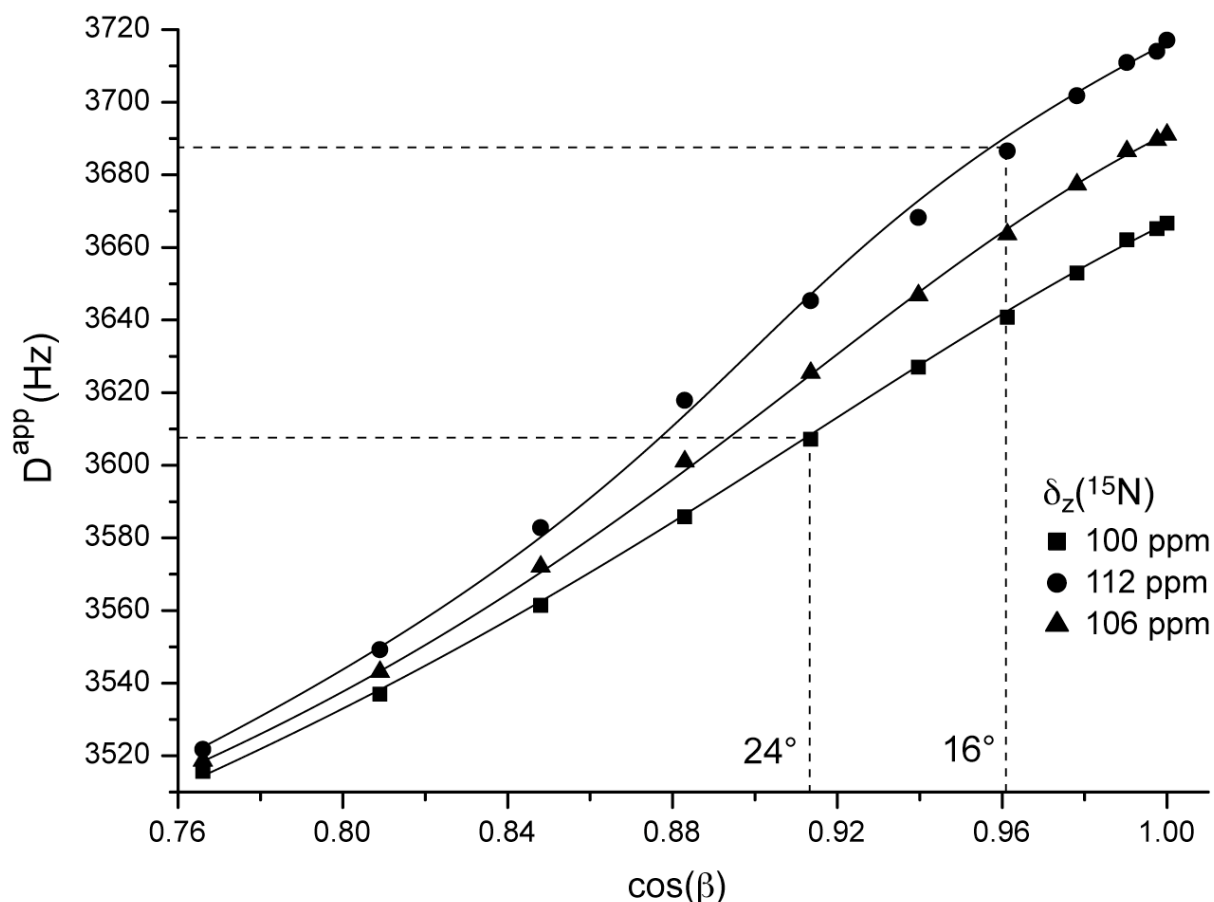
Supporting Figure 6. Dependence of the experimental dipolar splitting D^{app} on the ^{15}N anisotropic chemical shift δ_z and the angle β between the principal axis of the CSA tensor and the ^1H-^{15}N dipolar tensor. Typical values for the anisotropy of the ^{15}N chemical shift are $\Delta\delta=\delta_\parallel-\delta_\perp=170\pm8$ ppm or $\delta_z=106\pm6$ ppm.[3-7] The tensor of the ^{15}N-^1H heteronuclear dipolar coupling is collinear with the ^{15}N-^1H bond and tilted approximately by around 20° with respect to the principal axis of the ^{15}N CSA tensor.[3, 5] The simulation was carried out using the program SIMPSON.[2] The error induced by the uncertainty of the ^{15}N CSA tensor parameters is the major determinant of the error in the ^1H-^{15}N CPPI dipolar recoupling experiment. Assuming a variation of $\Delta\delta_z = \pm 6$ ppm around $\delta_z = 106$ ppm and $\Delta\beta = \pm4°$ around $\beta_0 = 20°$ yields an error of ± 35 Hz for the experimental dipolar splitting D^{app}. Assuming a scaling factor $\kappa = 0.359$ for the CPPI experiment, this translates into an uncertainty in the determination of the ^1H-^{15}N dipolar coupling in the order of ± 111 Hz. This error is added on top of the error that is obtained in the analysis of the experimental CPPI spectra as shown in Supporting Figure 3.

A)

^{15}N isotropic shift = 0.0 ppm
^{15}N isotropic shift = 12.5 ppm

B)

$\eta(^{15}N) = 0.0$
$\eta(^{15}N) = 0.2$
$\eta(^{15}N) = 0.5$

C)

$\delta_z(^1H) = 10$ ppm
$\delta_z(^1H) = 0$ ppm

D)

$\eta(^1H) = 0.00$
$\eta(^1H) = 0.65$
$\eta(^1H) = 1.00$
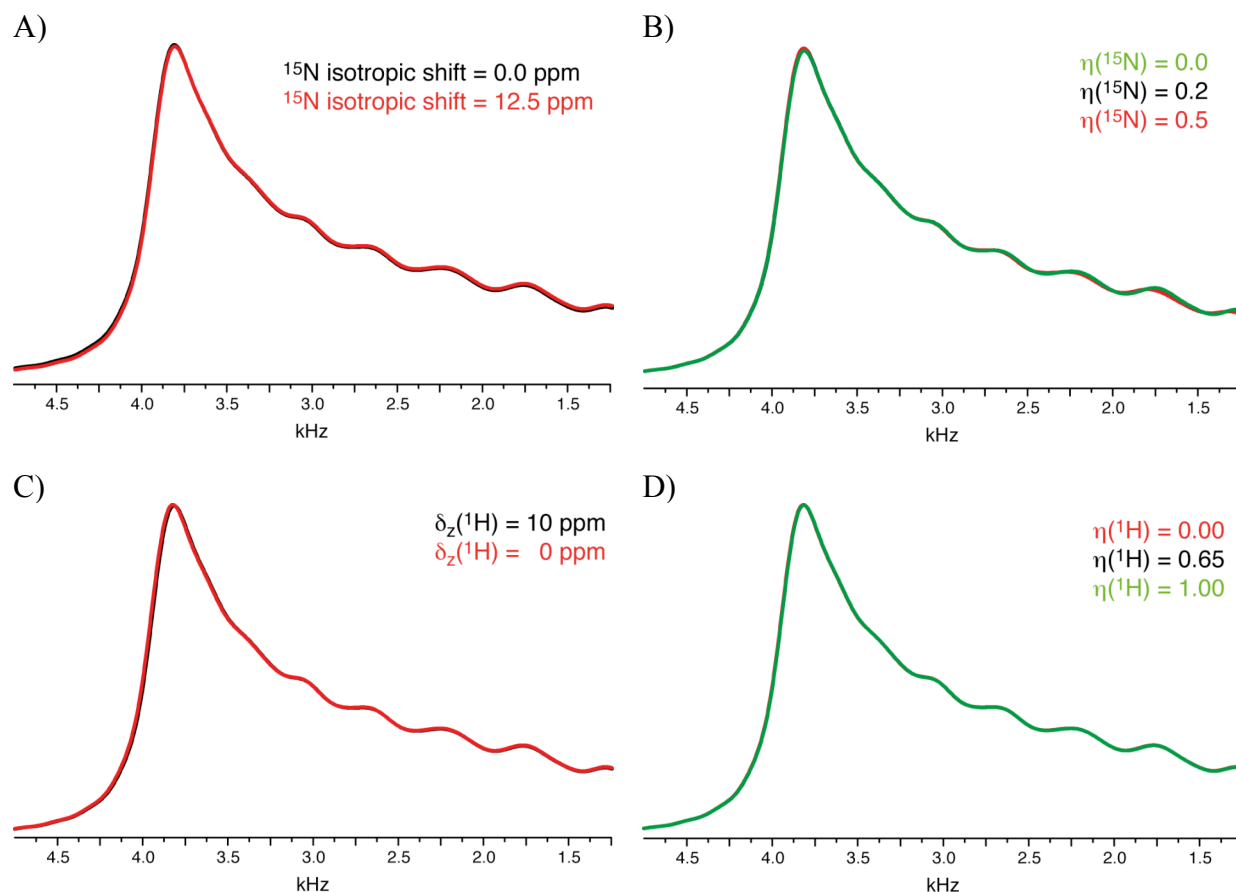
Supporting Figure 7. Influence of the isotropic ^{15}N chemical shift (A), the asymmetry η of the ^{15}N CSA tensor (B), the ^{1}H chemical shift anisotropy (C) and the ^{1}H chemical shift asymmetry η (D) on the apparent dipolar coupling D^{app} in the CPPI experiment. The simulations are carried out using the program SIMPSON.[2] We find virtually no influence of these parameters on the value of the extracted dipolar coupling.

Reference
Additional proton with
d(^{15}N,^{1}H) = 2.5 kHz
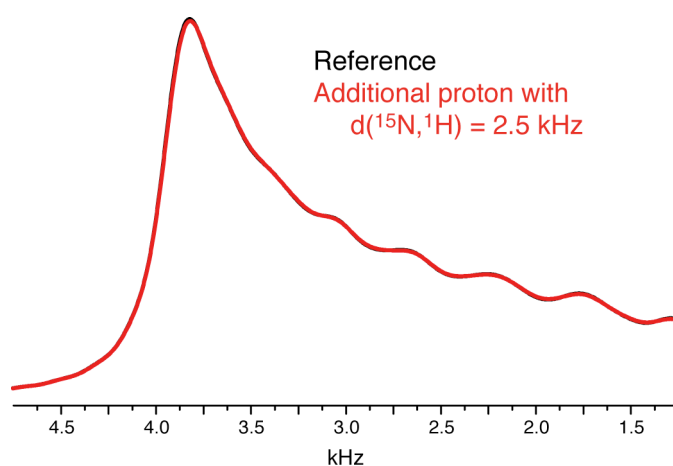
Supporting Figure 8. CPPI dipolar recoupling spectrum assuming that a second proton is interacting with the ^{15}N nucleus. The simulations are carried out using the program SIMPSON.[2] The influence of the remote proton is not observable in the simulation.
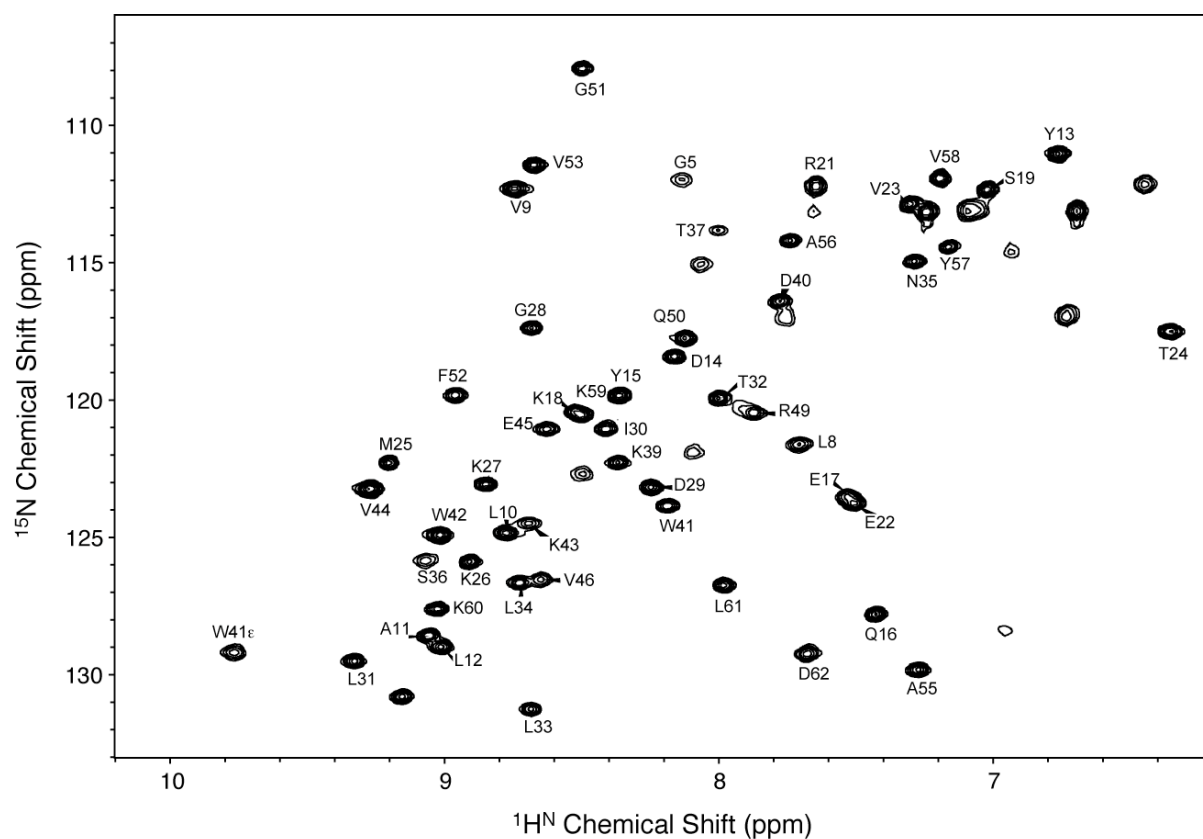
Supporting Figure 9. ^{1}H,^{15}N correlation experiment obtained for the α-spectrin SH3 domain in the solid-state. Only those resonances are taken into account the above described analysis which are well resolved in the 2D corrlation spectrum.
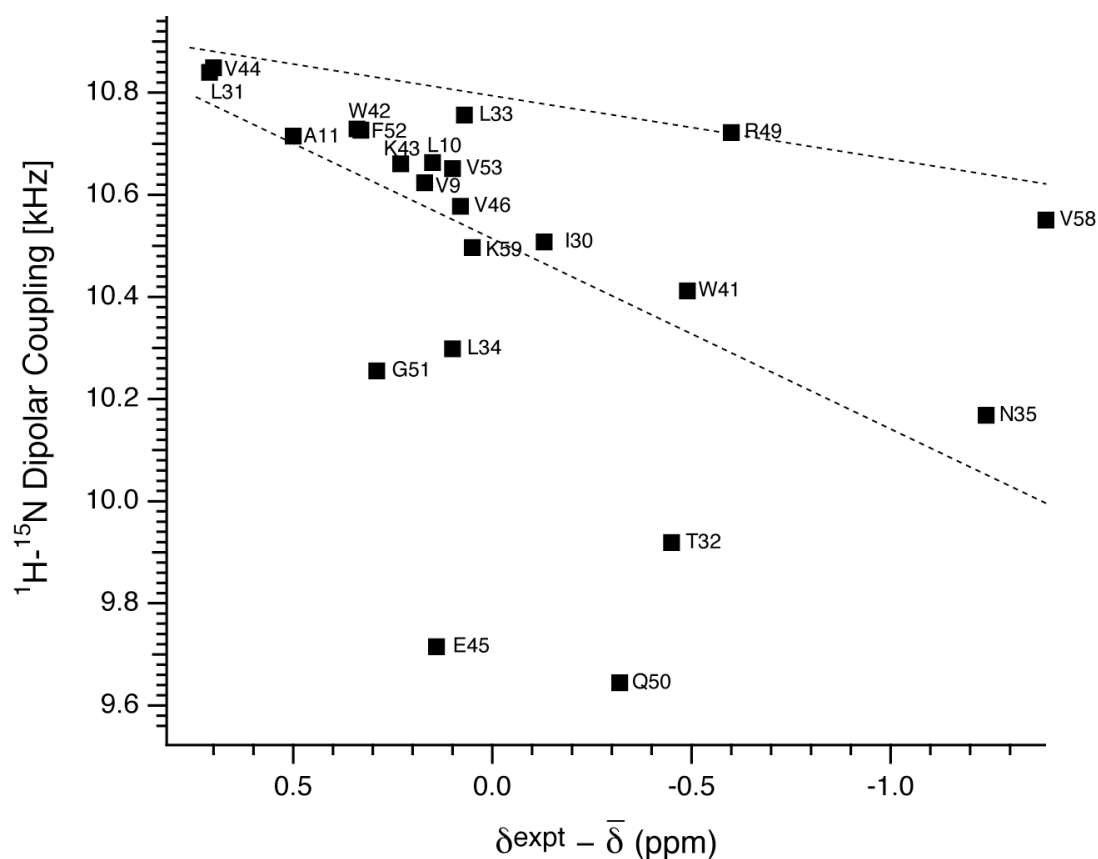
Supporting Figure 10. Correlation between the ^{1}H,^{15}N dipolar couplings as a function of the chemical shift difference between the experimental ^{1}H^N chemical shifts and the average H^N chemical shifts as given in the BMRB (http://www.bmrb.wisc.edu/ published/ ikura_cs_study/ part1.html). Only β-sheet residues are included in the plot. A similar trend as in the case of isotropic chemical shifts (Figure 3) is observed. We find the largest dipolar couplings for residues which are downfield shifted.

Supporting Table 1. ^{1}H,^{15}N dipolar couplings, ^{1}H^N and ^{15}N chemical shifts for α-spectrin SH3 in the solid-state.

Res	^{1}H,^{15}N dipolar coupling (Hz)	Error (Hz)	^{1}H^N chemical shift (ppm)	^{15}N chemical shift (ppm)
L8	9282,29	750	7,82	120,88
V9	10623,37	375	8,89	111,63
L10	10726,50	300	8,92	124,17
A11	10714,66	225	9,20	127,86
L12	10651,00	225	9,15	128,22
Y13	10354,72	225	6,91	110,35
D14	10194,39	250	8,31	117,71
Y15	10420,94	300	8,50	119,14
Q16	10113,82	300	7,57	127,09
E17	10235,21	300	7,68	122,89
K18	10520,61	150	8,67	119,74
S19	9814,97	450	7,17	111,63
R21	7286,64	750	7,79	111,55
E22	10507,62	450	7,65	122,93
V23	10280,31	450	7,47	112,29
T24	9299,35	900	6,49	116,89
M25	10696,45	225	9,25	121,63
K26	10516,03	225	9,06	125,23
K27	10457,69	300	8,99	122,40
G28	10654,06	225	8,83	116,72
I30	10496,50	300	8,58	120,24
L31	10839,76	450	9,48	128,83
T32	9918,71	900	8,14	119,21
L33	10755,85	375	8,84	130,54
L34	10577,50	450	8,87	125,98
N35	10167,96	375	7,44	114,20
T37	10562,41	900	8,18	112,97
K39	10128,61	450	8,52	121,54
D40	10514,35	375	7,94	115,69
W41	10411,66	375	8,34	123,22
W42	10662,98	225	9,16	124,17
K43	10660,54	300	8,83	123,76
V44	10848,73	300	9,42	122,59
E45	10507,79	300	8,78	120,31
V46	10298,28	450	8,80	125,77
R49	9714,97	300	8,02	119,83
Q50	9644,18	300	8,26	117,10
G51	10255,16	225	8,64	107,25
F52	10728,84	225	9,11	119,11
V53	10651,12	300	8,82	110,82
A55	10533,34	150	7,43	129,17
A56	10399,06	225	7,90	113,56
Y57	10395,95	325	7,31	113,75
V58	10550,32	300	7,33	111,26
K59	10366,96	300	8,65	119,87

K60	10219,36	225	9,16	126,87
L61	9961,91	225	8,12	126,06
D62	6780,00	750	7,83	128,60

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