Supporting Information for

^{1}H-^{13}C/^{1}H-^{15}N Heteronuclear Dipolar Recoupling by R-Symmetry Sequences Under Fast Magic Angle Spinning for Dynamics Analysis of Biological and Organic Solids

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Table S1. Summary of R-symmetry sequences suitable for heteronuclear dipolar recoupling under fast MAS conditions, $10 \leq N \leq 30$, and $1 < N/2n \leq 5.0$*.

N	n	v	ω_1/ω_R	K_{sc}
10	3	1	1.667	0.2236
10	1	3	5	0.3064
12	5	4	1.2	0.1550
14	2	3	3.5	0.2946
14	3	1	2.333	0.2669
14	4	1	1.75	0.2314
14	5	3	1.4	0.1907
14	6	5	1.167	0.1479
16	3	2	2.667	0.2785
16	5	2	1.6	0.2165
16	7	6	1.143	0.1425
18	2	5	4.5	0.3038
18	4	1	2.25	0.2634
18	5	1	1.8	0.2356
18	7	5	1.286	0.1718
18	8	7	1.125	0.1384
20	3	4	3.333	0.292
20	7	4	1.429	0.1949
20	9	8	1.111	0.1351
22	3	5	3.667	0.2967
22	4	3	2.75	0.2808
22	5	1	2.2	0.2610
22	6	1	1.833	0.2383
22	7	3	1.571	0.2134
22	8	5	1.375	0.1869
22	9	7	1.222	0.1597
22	10	9	1.1	0.1325
24	5	2	2.4	0.2697
24	7	2	1.714	0.2281
24	11	10	1.091	0.1303
26	3	7	4.333	0.3028
26	4	5	3.25	0.2910
26	5	3	2.6	0.2764
26	6	1	2.167	0.2593
26	7	1	1.857	0.2402

26	8	3	1.625	0.2193
26	9	5	1.444	0.1971
26	10	7	1.3	0.1742
26	11	9	1.182	0.1512
26	12	11	1.083	0.1283
28	3	8	4.667	0.3048
28	5	4	2.8	0.2820
28	9	4	1.556	0.2115
28	11	8	1.273	0.1694
28	13	12	1.077	0.1268
30	4	7	3.75	0.2977
30	7	1	2.143	0.2581
30	8	1	1.875	0.2414
30	11	7	1.364	0.1850
30	13	11	1.154	0.1451
30	14	13	1.071	0.1254
4	1	4	2	0.5

* N, *n*, and *v* are the symmetry numbers associated with each sequence. w_1 and w_R denote the RF field strength and the MAS frequency, respectively. K_{sc} is the effective scaling factor of the dipolar lineshape recoupled by a specific symmetry sequence.

Table S2. ^{1}H-$^{13}C^{\alpha}$ dipolar coupling constants for each residue in the CAP-Gly domain of dynactin, extracted from the 3D $R16_3^2$ DIPSHIFT experiment conducted at the MAS frequency of 40 kHz.

Residue #	^{1}H-^{13}C dipolar coupling (kHz)	χ^2	Secondary structure
L27	19.6 ± 0.3	0.59	Loop
S31	20.5 ± 0.2	0.51	Loop
I36	19.3 ± 0.2	1.51	Loop
R41	21.2 ± 0.3	1.03	β-Sheet
G42	20.8 ± 0.2	0.54	β-Sheet
T43	20.8 ± 0.2	0.46	β-Sheet
V44	20.7 ± 0.4	2.95	β-Sheet
V47	21.2 ± 0.2	0.39	β-Sheet
G48	20.6 ± 0.2	0.81	β-Sheet
A49	19.4 ± 0.2	1.48	Terminus of β-Sheet
T50	20.2 ± 0.2	0.83	Loop
L51	20.0 ± 0.3	2.07	Loop
T54	19.2 ± 0.2	1.82	Loop
K56	19.2 ± 0.2	0.62	Turn
V60	20.8 ± 0.2	0.74	β-Sheet
I61	20.7 ± 0.4	2.54	β-Sheet
L62	19.3 ± 0.5	3.45	Terminus of β-Sheet
D63	19.0 ± 0.4	1.72	Loop
A65	21.0 ± 0.2	0.68	Loop
K68	19.2 ± 0.3	2.89	Loop
N69	19.9 ± 0.2	0.26	Loop
D70	19.9 ± 0.2	0.29	Loop
T72	21.2 ± 0.2	0.20	β-Sheet
V73	19.5 ± 0.3	2.14	Loop
Q74	19.2 ± 0.3	1.66	Loop
R76	20.7 ± 0.2	0.65	β-Sheet
K77	19.4 ± 0.2	0.39	Terminus of β-Sheet
T80	20.0 ± 0.4	3.16	Loop
C81	19.2 ± 0.2	1.09	Loop
D82	19.2 ± 0.2	0.48	Loop
E83	19.3 ± 0.3	1.76	Loop
H85	19.4 ± 0.6	4.04	Loop
I87	20.9 ± 0.3	2.05	β-Sheet
V89	18.9 ± 0.2	0.58	Terminus of β-Sheet
Q91	19.2 ± 0.2	1.76	Loop
Q93	19.2 ± 0.3	1.67	Loop
I94	21.8 ± 0.2	1.11	β-Sheet

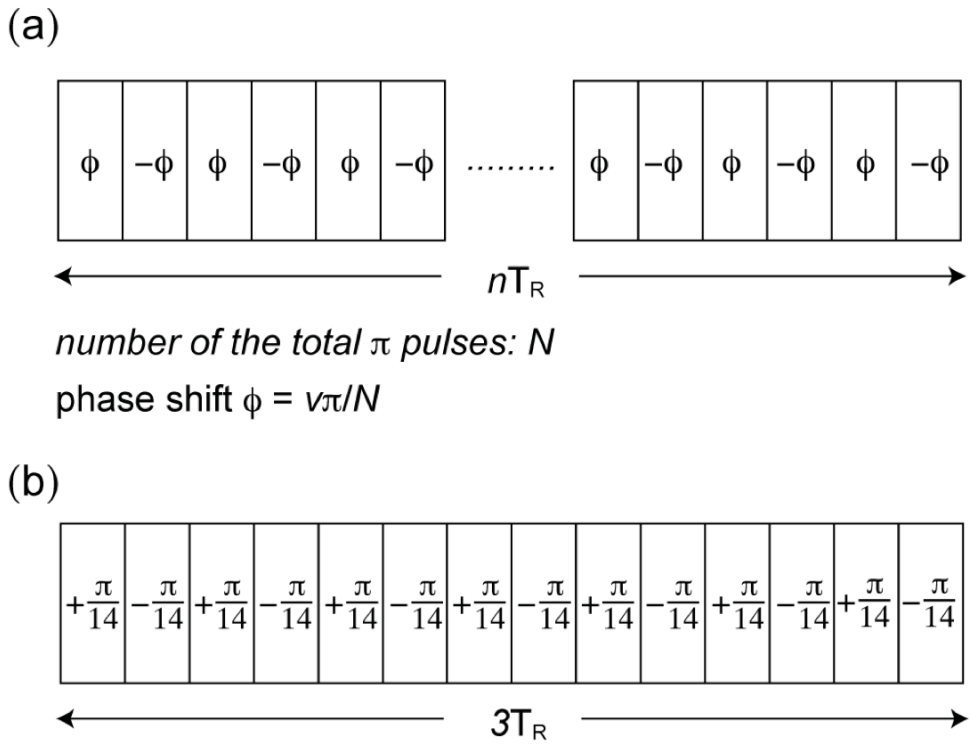

Figure S1. (a) Schematic representation of RN_n^v symmetry sequences. Each square denotes one π pulse, and ϕ denotes the phase of π pulse. (b) An example of R-type symmetry sequence, $R14_3^1$. In this sequence, 14 π pulses are contained in 3 rotor periods; the phase is ± π/14, and the rf field strength is $7/3\omega_R$.

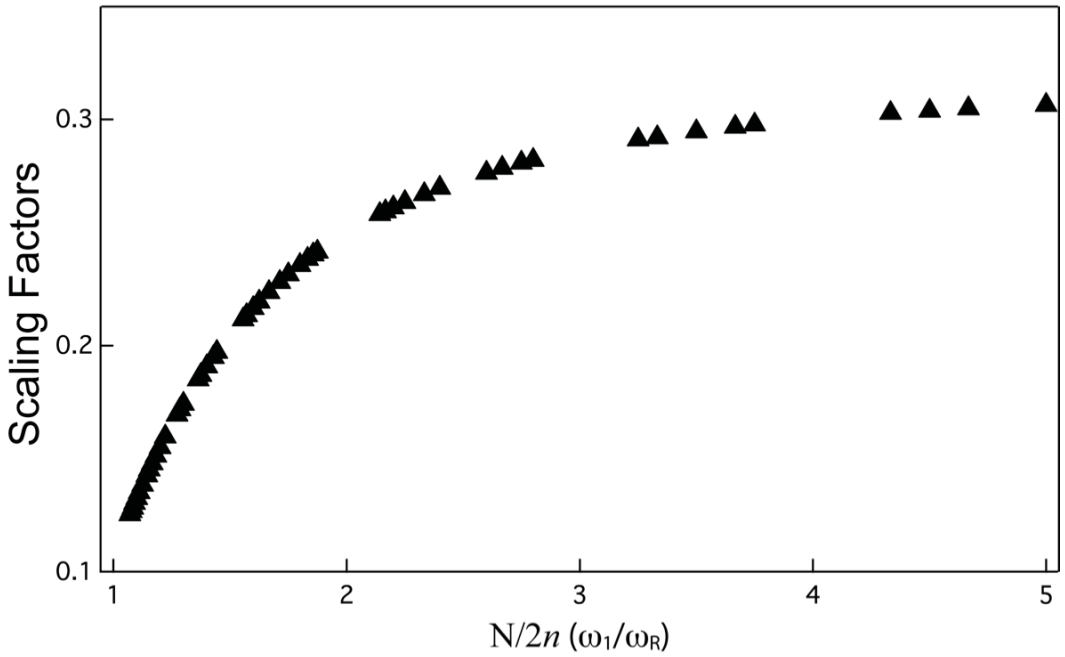

Figure S2. Effective scaling factors for γ-encoded dipolar recoupling RN_n^v symmetry sequences plotted as a function of the ratio of the symmetry numbers, N/2*n*. π pulses were used as the basic R elements, and the corresponding symmetry sequences are listed in Table *S1*. The ratio of N/2*n* determines the required rf field strength, $\omega_1 = N\omega_R/2n$.

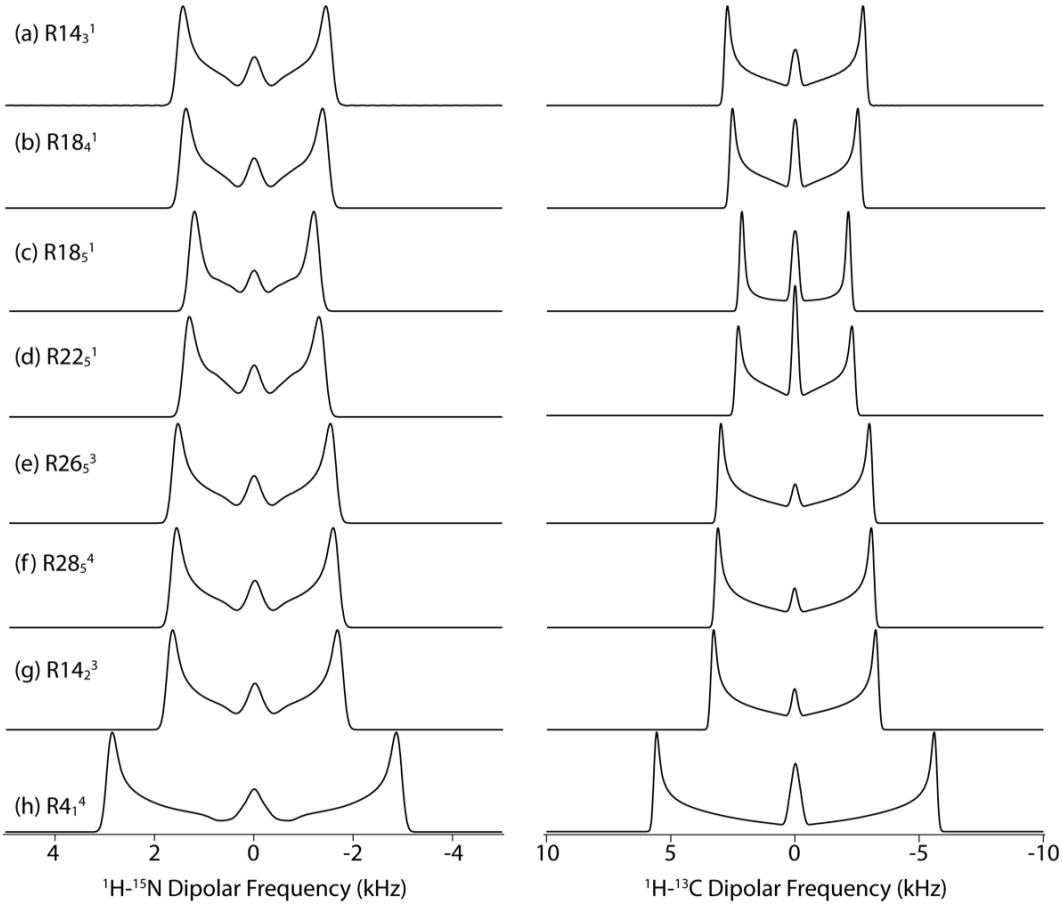

Figure S3. Simulated ^{1}H-^{15}N (left) and ^{1}H-^{13}C (right) dipolar lineshapes recoupled by (a) R14$_3^1$, (b) R18$_4^1$, (c) R18$_5^1$, (d) R22$_5^1$, (e) R26$_5^3$, (f) R28$_5^4$, (g) R14$_2^3$, and (h) R4$_1^4$ symmetry sequences at the MAS frequency of 40 kHz. π pulses were used as the basic R elements, and the applied RF field strengths were 93.3, 90, 72, 88, 104, 112, 140, and 80 kHz in (a)-(h), respectively. Isolated H-N and H-C spin pairs were used for ^{1}H-^{15}N and ^{1}H-^{13}C RN$_n^v$ dipolar simulations, respectively. The one-bond heteronuclear dipolar couplings were 11.8 kHz for ^{1}H-^{15}N and 22.7 kHz for ^{1}H-^{13}C. ^{1}H CSA parameters were: i) δ_o = 10.0 ppm and η =1 for the NH proton, and ii) δ_o = 2.0 ppm and η =1 for the CH proton. The δ_{zz} principal component of the CSA tensor was assumed to lie along the dipolar vector. 986 ZCW (α, β) orientations and 3 γ angles were used to generate a powder average for all γ-encoded RN$_n^v$ symmetry sequences, except for the non-γ encoded R4$_1^4$, where the same ZCW angle set and 64 γ angles were employed.

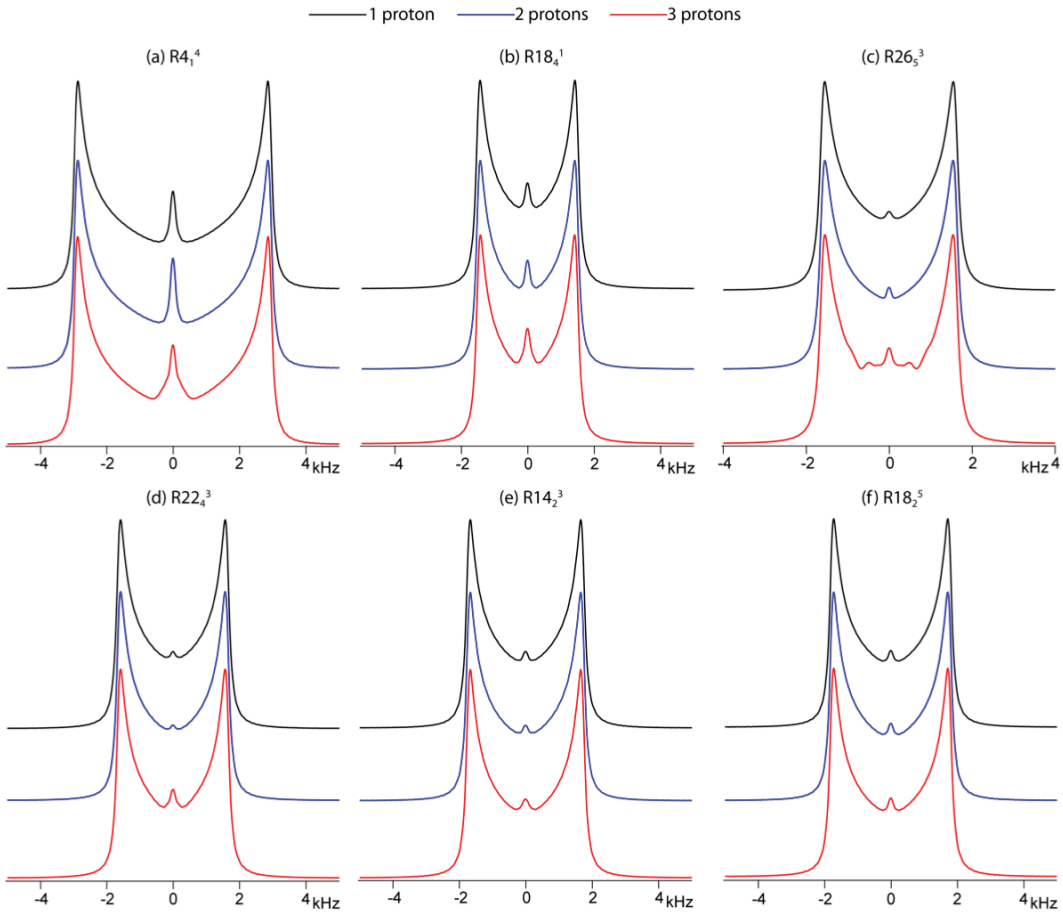

Figure S4. Simulated ^{1}H-^{15}N dipolar lineshapes for isolated NH (black), NH_2 with one remote proton (blue) and NH_3 with two remote protons (red) spin systems, recoupled by (a) $R4_1^4$, (b) $R18_4^1$, (c) $R26_5^3$, (d) $R22_4^3$, (e) $R14_2^3$, and (f) $R18_2^5$ symmetry sequences at a MAS frequency of 40 kHz. π pulses were used as the basic R elements, and the applied RF field strengths were 80, 90, 104, 110, 140, and 180 kHz in (a)-(f), respectively. All simulation parameters except the spin system were same as Figure *S3*, and the geometry was i) $d_{N\text{-}H}$ = 1.01 Å for NH spin system; ii) $d_{N\text{-}H1}$ = 1.01 Å, $d_{N\text{-}H2}$ = 2.48 Å, and $d_{H1\text{-}H2}$ = 2.64 Å for NH_2 spin system; iii) $d_{N\text{-}H1}$ = 1.01 Å, $d_{N\text{-}H2}$ = 2.48 Å, $d_{N\text{-}H3}$ = 2.48 Å, $d_{H1\text{-}H2}$ = 2.64 Å, $d_{H1\text{-}H3}$ = 2.64 Å, and $d_{H2\text{-}H3}$ = 1.96 Å for NH_3 spin system.

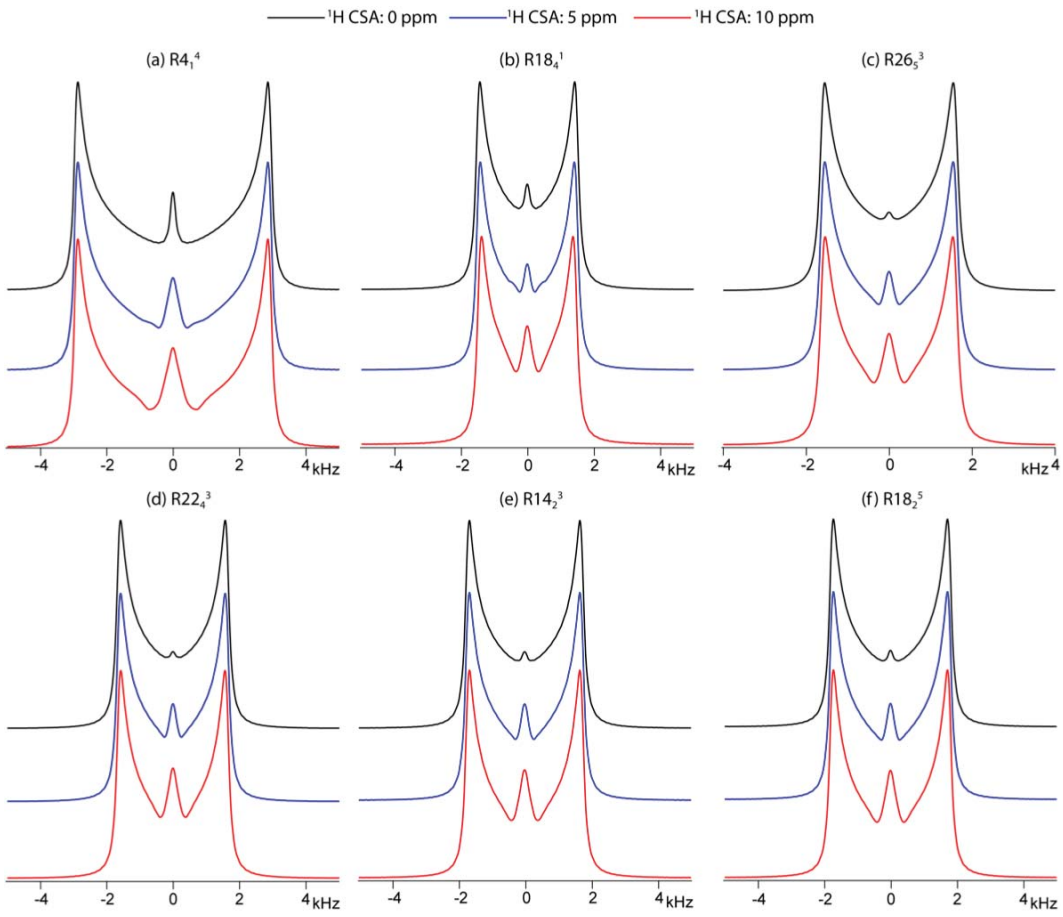

Figure S5. Simulated ^{1}H-^{15}N dipolar lineshapes for isolated N-H spin system containing amide proton CSA, with reduced shift anisotropy $\delta_{\circ}$ of 0 ppm (black), 5 ppm (blue) and 10 ppm (red), recoupled by (a) $R4_1^4$, (b) $R18_4^1$, (c) $R26_5^3$, (d) $R22_4^3$, (e) $R14_2^3$, and (f) $R18_2^5$ symmetry sequences at the MAS frequency of 40 kHz. All simulation parameters except spin systems are the same as in Figure *S4*.

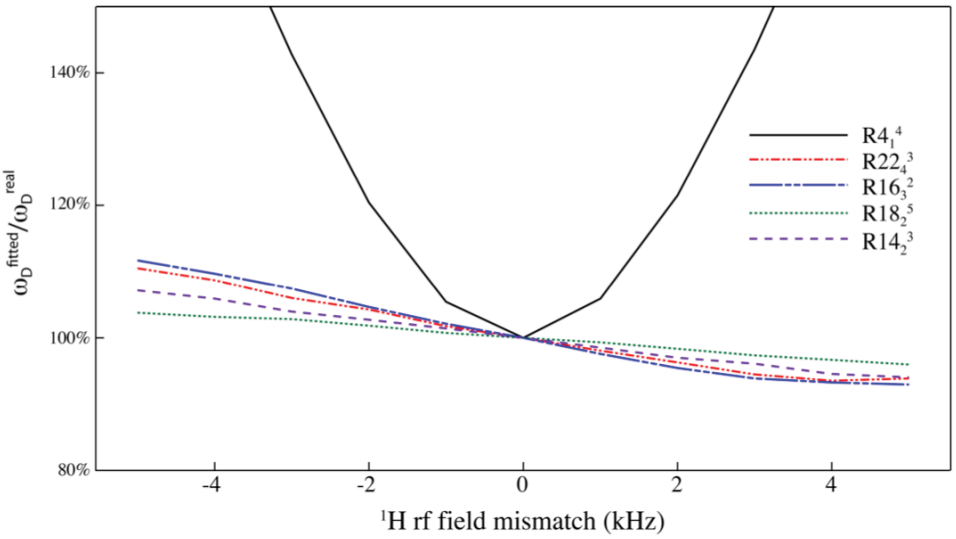

Figure S6. The influence of the mismatch of ^{1}H rf field strength on the accuracy of the ^{1}H-^{15}N dipolar measurement with various symmetry sequences, $R22_4^3$ (red), $R16_3^2$ (blue), $R18_2^5$ (green), and $R14_2^3$ (purple). All simulation parameters except the rf field strength are the same as in Figure *S4*.

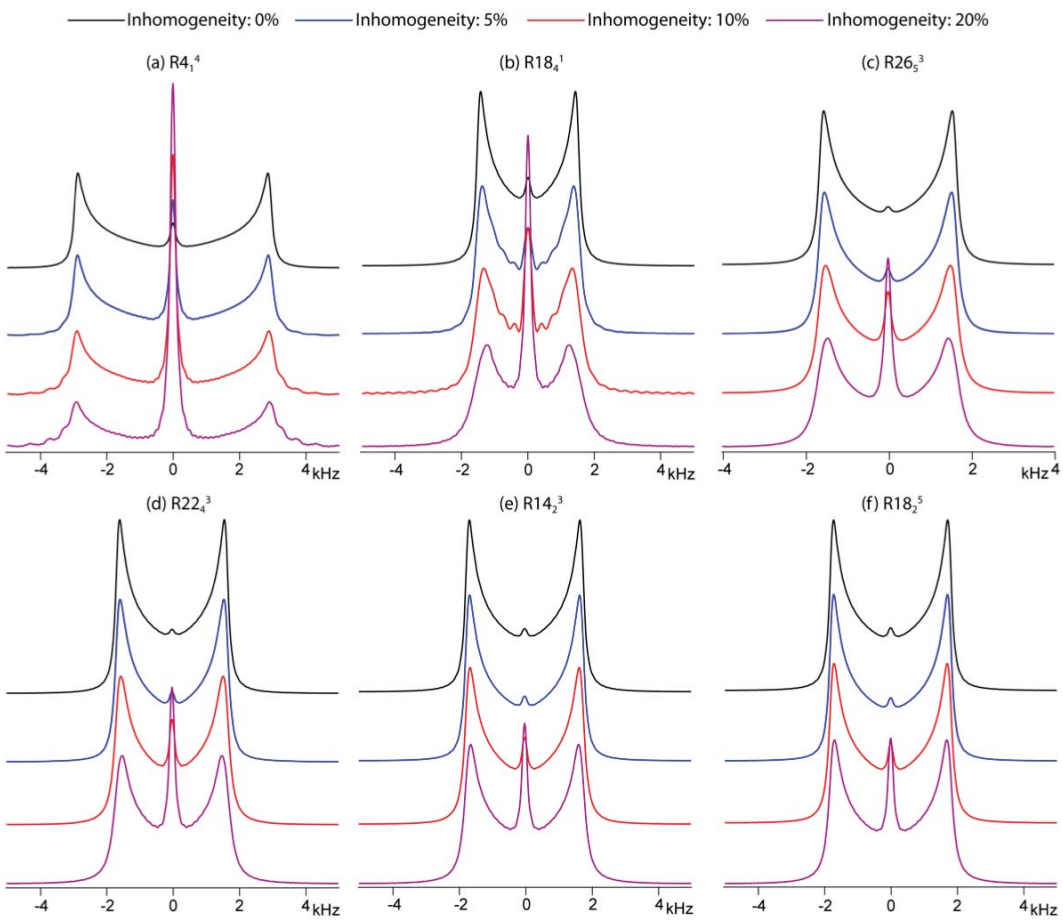

Figure S7. Simulated ^{1}H-^{15}N dipolar lineshapes for isolated NH spin system with rf field homogeneity of 100% (black), 95% (blue), 90% (red) and 80% (purple), recoupled by (a) $R4_1^4$, (b) $R18_4^1$, (c) $R26_5^3$, (d) $R22_4^3$, (e) $R14_2^3$, and (f) $R18_2^5$ symmetry sequences at the MAS frequency of 40 kHz. Lorentzian distribution was employed to simulate the effect of the RF field inhomogeneity. All other simulation parameters are the same as in Figure *S4*.

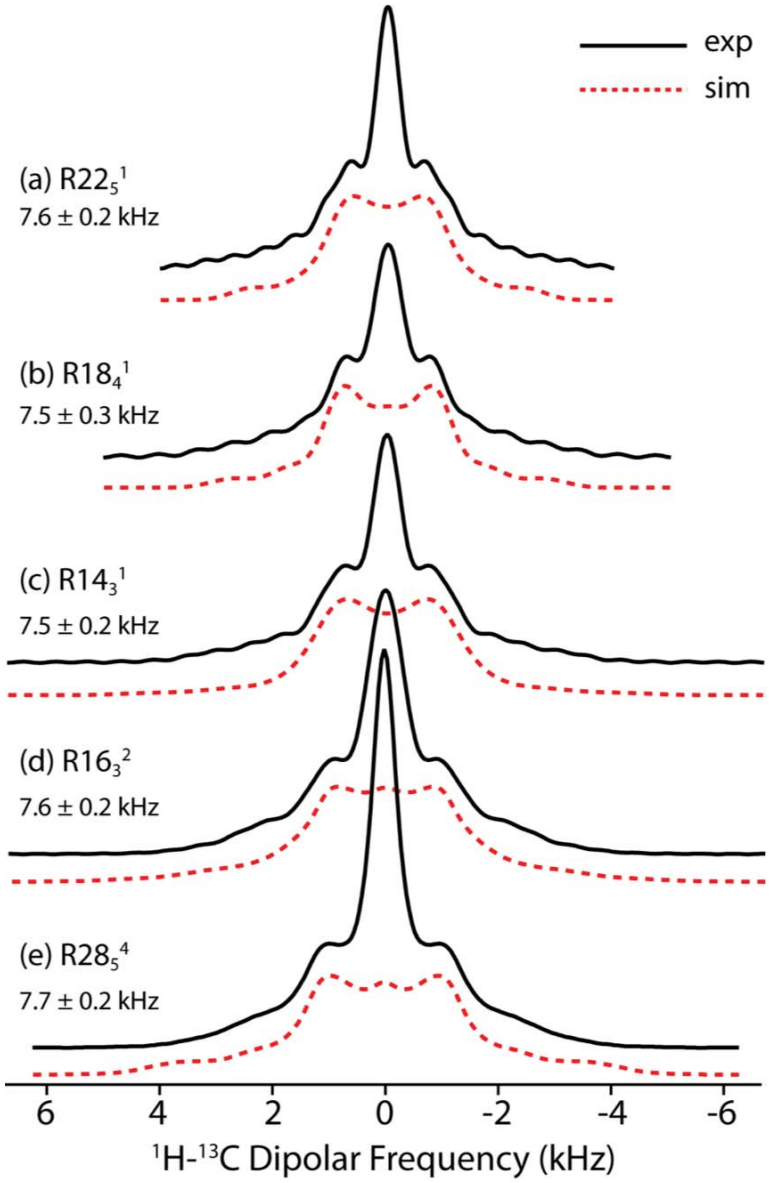

Figure S8. Black solid lines: experimental ^{1}H-^{13}C dipolar lineshapes of the CH_3 groups in U-^{13}C,^{15}N-alanine recorded at the MAS frequency of 40 kHz using (a) R22$_5^1$, (b) R18$_4^1$, (c) R14$_3^1$, (d) R16$_3^2$, and (e) R28$_5^4$ symmetry sequences. During the dipolar evolution period t_1, the ^{1}H RF field irradiation strength is 88, 90, 93.3, 106.7, and 112 kHz for (a)-(e), respectively. Red dashed lines: simulated best-fit ^{1}H-^{13}C dipolar lineshapes. The dipolar coupling constants extracted from the experimental lineshapes are indicated next to each spectrum.

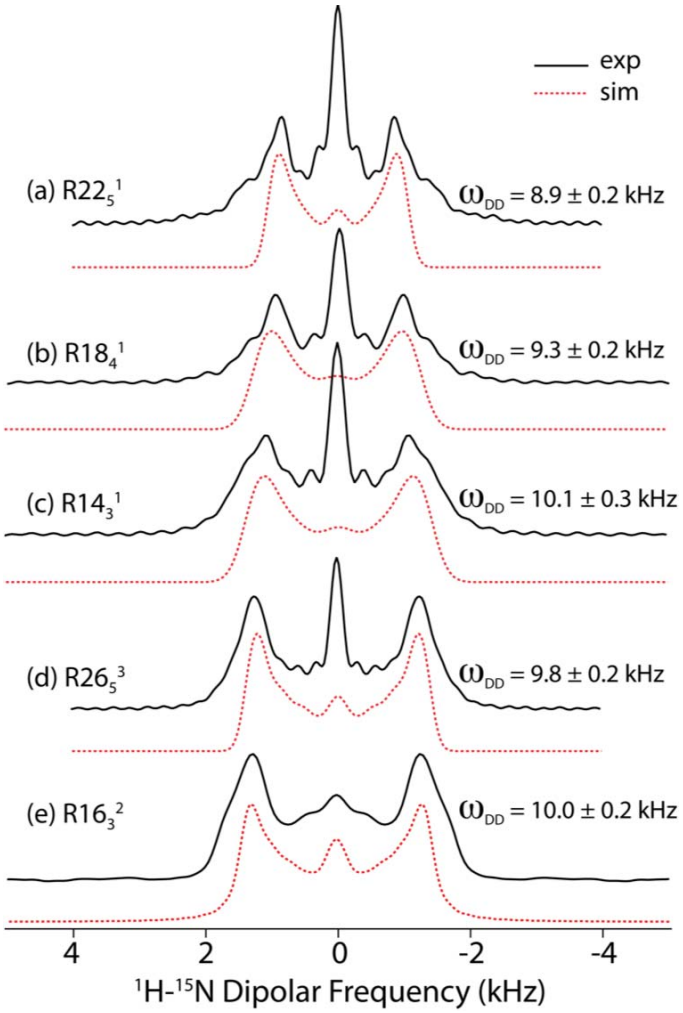

Figure S9. Solid lines: Experimental ^{1}H-^{15}N dipolar lineshapes in [^{15}N]-N-acetyl-valine (NAV) recorded at the MAS frequency of 40 kHz, using (a) R22$_5^1$, (b) R18$_4^1$, (c) R14$_3^1$, (d) R26$_5^3$ and (e) R16$_3^2$ symmetry sequences. The ^{1}H rf field strength applied during the symmetry pulse irradiation was 88, 90, 93, 104, and 106.7 kHz for (a)-(e), respectively. Dotted lines: Simulated best-fit ^{1}H-^{15}N dipolar patterns. The dipolar coupling constants extracted from the experimental lineshapes are indicated next to each spectrum.

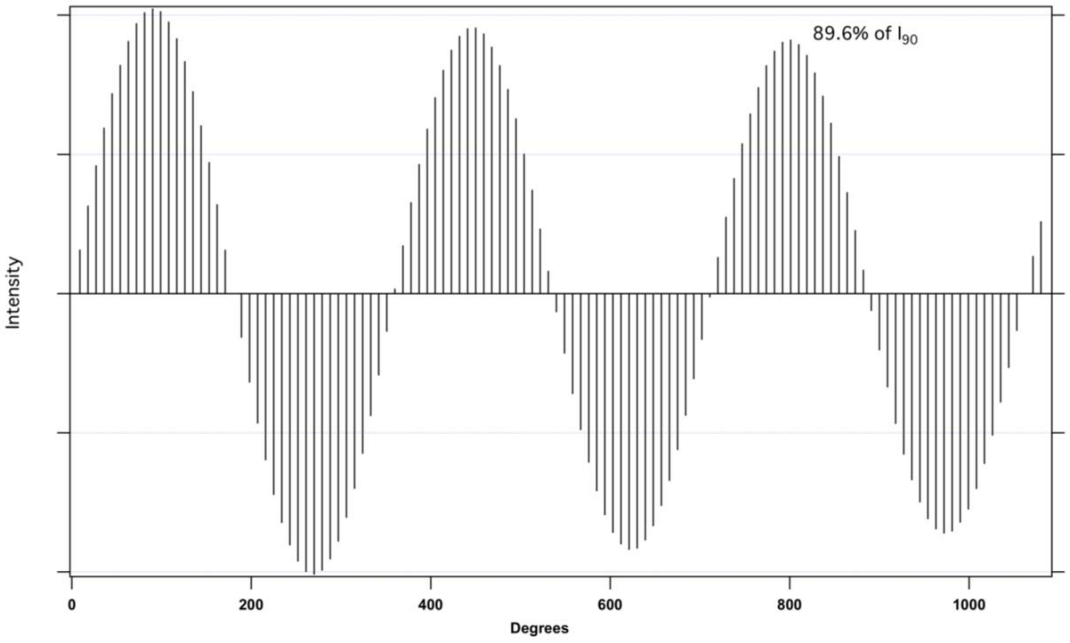

Figure S10. Nutation profile of the ^{15}N CP magnetization of N-acetyl-valine (NAV) in our 1.8 mm fast MAS probe, The signal intensity is plotted as a function of the ^{1}H excitation pulse angle. The rf field homogeneity measured as I_{810}/I_{90}, is 89.6%.

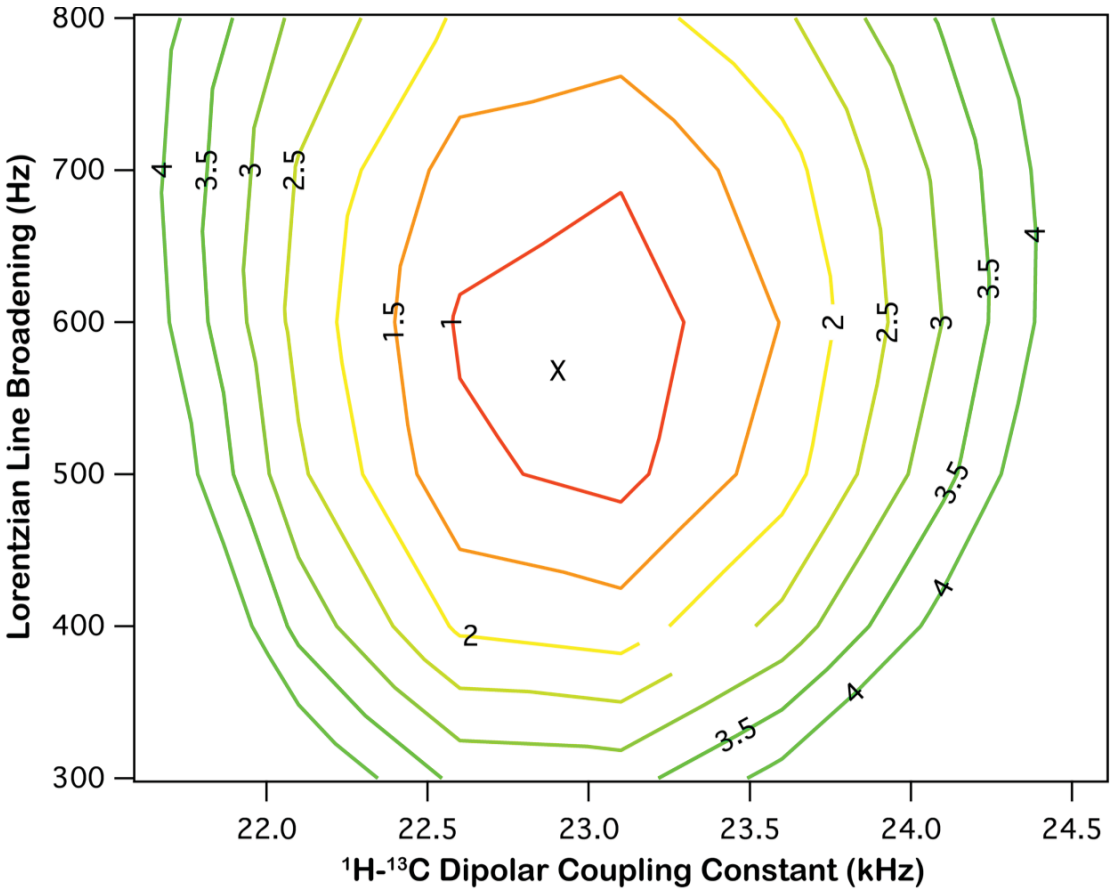

Figure S11. Contour plot for the least square error criterion χ^2 from the Simplex fitting of ^{1}H-$^{13}C^{\alpha}$ dipolar lineshape of Tyr 164 in [U-^{13}C, ^{15}N]-Tyr HIV-1 C-terminal domain (CTD) of CA protein, recorded by $R16_3^2$ at the MAS frequency of 40 kHz, as a function of dipolar coupling constant (ω_D) and Lorentzian line broadening (LB). The best-fit parameters are ω_D = 22.8 ± 0.3 kHz and LB = 573 ± 112 Hz, indicated by the cross symbol.

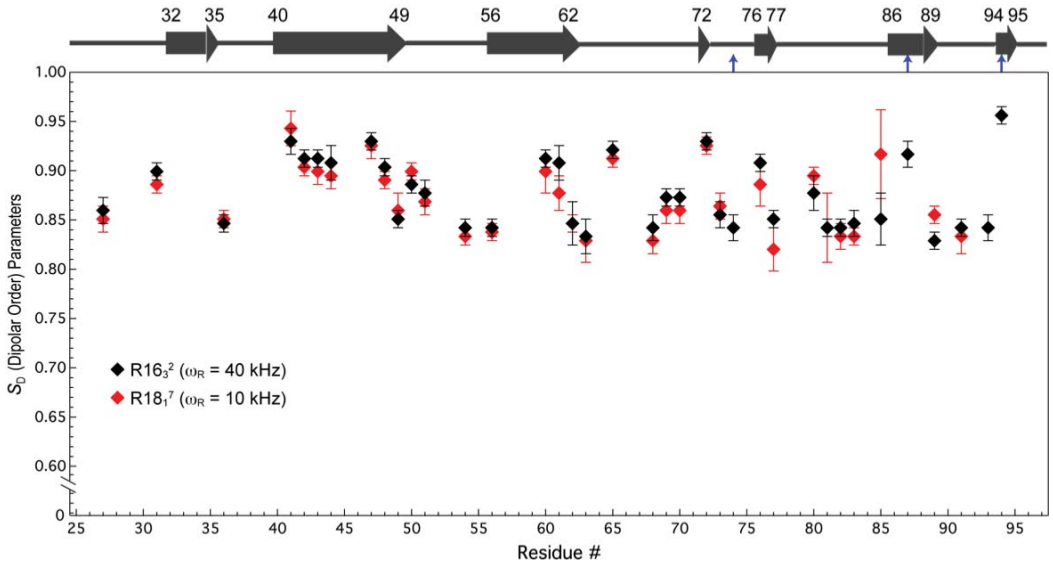

Figure S12. Overlay of the experimental H-C$^{\alpha}$ dipolar order parameters plotted as a function of the residue number for the sparsely-^{13}C/U-^{15}N enriched CAP-Gly domain of dynactin, recorded by R16$_3^2$ symmetry sequence at the MAS frequency of 40 kHz (black), and by R18$_1^7$ symmetry sequence at the MAS frequency of 10 kHz (red). Ignoring the missing residue (marked as blue arrow bars) in the R18$_1^7$-based DIPSHIFT measurement, the average deviation in the derived dipolar order parameter between the two measurements is 0.012, corresponding to the deviation in the dipolar coupling constant of 270 Hz. The excellent agreement between these measurements demonstrates that the heteronuclear dipolar coupling measurements by R-type symmetry sequences at fast MAS frequencies are accurate and robust.