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Supporting Information

Ms No: JA981686M-25-71/Revised,
David Fushman, Nico Tjandra, David Cowburn "Direct measurement of ^{15}N chemical shift anisotropy in solution."

Derivation of ^{15}N CSA and θ from η/R_2 data at two fields.

Since $\{c \propto B_0\}$ is the only field-dependent parameter in the right-hand side of the Equation [4] for η/R_2, it is possible to determine the magnitude of ^{15}N CSA using a ratio of η/R_2 values acquired at different magnetic fields:

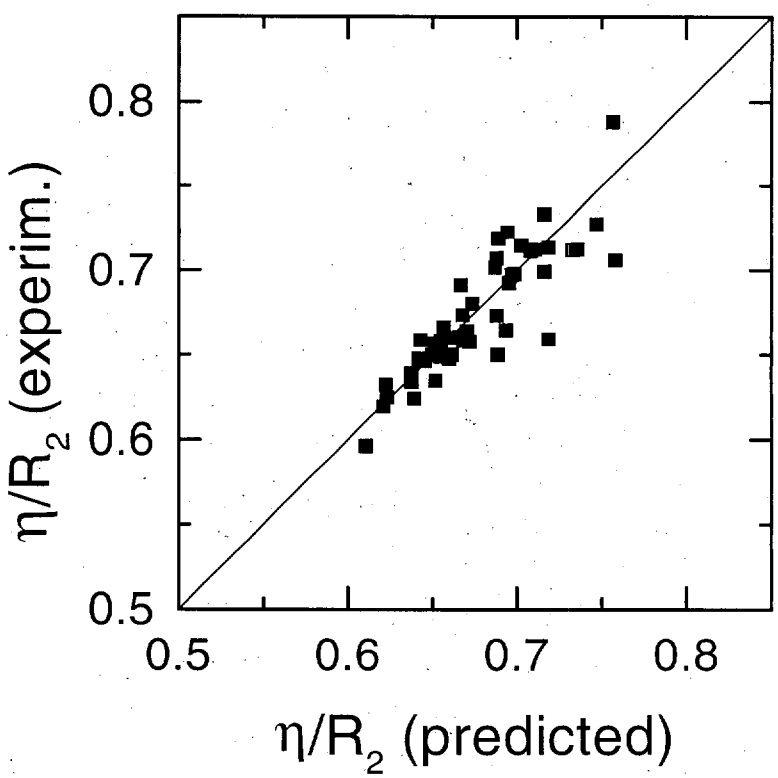
$$\sigma_{\parallel} - \sigma_{\perp} = -\frac{3d}{\omega_{NA}} \sqrt{\frac{(\omega_A/\omega_B)^2 - 1}{(\omega_A/\omega_B)(\eta_A/R_{2A})/(\eta_B/R_{2B}) - 1} - 1} \qquad [A1]$$

The subscripts here refer to $\omega_A/(2\pi)$=600 MHz, $\omega_{NA}/(2\pi)$=61 MHz, and $\omega_B/(2\pi)$=360 MHz, if, for example, the 600 and 360 MHz data are analyzed. This value of ^{15}N CSA can then be used to determine the θ angle:

$$\theta = \cos^{-1} \sqrt{\frac{1}{3}\left(1 + \frac{\eta}{R_2}\frac{d^2 + c^2}{dc}\right)} \qquad [A2]$$

For a given pair of η/R_2 values at two fields, these equations describe the intersection of the corresponding curves in terms of CSA and θ, as illustrated in Figure 1b (see text). Supplementary Figure shows good agreement between the experimental and predicted values of η/R_2 at 500 MHz.

Supplementary Figure. Agreement between measured and predicted values of η/R_2 at 500 MHz. The predicted values were calculated according to Equation [4] using the CSA and θ values derived from the pair-wise analysis of η/R_2 data at 600 and 360 MHz, according to Equations [A1], [A2]. Shown are only those residues where the experimental data satisfy the inequalities listed in [Fushman, D.; Cowburn, D. *J.Am.Chem.Soc.* **1998**, *120*, 7109-10], including $[1+\eta/R_2\,(d^2+c^2)/dc]/3 = \cos^2\theta \leq 1$ (see Equation [A2]).



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Supporting Table. Summary of experimental data, η/R_2, at three fields, and the results of present analysis, ^{15}N CSA and angle θ, for human ubiquitin. Here χ^2 is the value of the target function, Equation [5], and $P(\chi^2)$ is the probability that the optimized value of the target function should exceed a particular χ^2 value by chance. Numbers in parentheses indicate standard errors in the corresponding parameters. A 95% confidence limits for the goodness of fit were imposed here, i.e. those residues with $P(\chi^2)<0.05$, marked with an asterisk, are not shown in Figures 4b,c, 5, and 7, nor were these residues used for CSA-θ statistical analysis. Those θ values which are ill-determined because of large estimated errors, indicated by 'x', are not shown in Figures 4c and 5. Residues Ile^{23} and Asn^{25} were not included in the analysis because of significant conformational exchange contribution [Fushman, D.; Cowburn, D. *J.Am.Chem.Soc.* **1998**, *120*, 7109-10.].

Res#	η/R_2 360 MHz	η/R_2 500 MHz	η/R_2 600 MHz	CSA, ppm	θ, deg	χ^2	$P(\chi^2)$
2	0.570 (0.010)	0.714 (0.011)	0.788 (0.006)	-170.22 (0.60)	15.60 (0.13)	0.003	0.956
3	0.505 (0.012)	0.635 (0.013)	0.728 (0.006)	-140.60 (11.42)	13.96 (5.25)	0.81	0.368
4	0.496 (0.012)	0.635 (0.013)	0.704 (0.006)	-156.96 (3.14)	19.69 (0.58)	0.05	0.821

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5	0.514	(0.013)	0.664	(0.012)	0.756	(0.007)	-132.96	(2.74)	6.40	(2.94)	0.04	0.845		
6	0.592	(0.012)	0.712	(0.012)	0.793	(0.006)	-182.97	(14.22)	17.28	(2.62)	1.51	0.219		
7	0.526	(0.010)	0.674	(0.010)	0.735	(0.006)	-167.23	(9.50)	19.03	(1.77)	0.77	0.380		
8	0.518	(0.010)	0.692	(0.009)	0.749	(0.007)	-151.71	(20.62)	14.52	(7.65)	4.79	0.029	*	
9	0.549	(0.012)	0.660	(0.010)	0.701	(0.007)	-215.54	(2.17)	25.21	(0.14)	0.03	0.862		
10	0.549	(0.010)	0.682	(0.009)	0.802	(0.008)	-138.98	(17.28)	0	(7.44)	6.32	0.012	*	
11	0.602	(0.011)	0.788	(0.009)	0.838	(0.008)	-173.43	(20.61)	10.62	(6.77)	6.60	0.010	*	
12	0.504	(0.010)	0.675	(0.010)	0.763	(0.007)	-130.57	(8.55)	0	(5.84)	1.29	0.257	x	
13	0.556	(0.011)	0.674	(0.011)	0.746	(0.006)	-183.89	(10.87)	20.57	(1.43)	0.88	0.348		
14	0.524	(0.011)	0.680	(0.011)	0.748	(0.006)	-156.51	(10.85)	16.13	(3.15)	0.75	0.386		
15	0.560	(0.014)	0.698	(0.013)	0.805	(0.007)	-142.23	(10.36)	0	(5.78)	1.58	0.209	x	
16	0.540	(0.010)	0.701	(0.011)	0.785	(0.007)	-142.78	(3.71)	7.12	(3.22)	0.12	0.728		
17	0.508	(0.012)	0.657	(0.012)	0.739	(0.006)	-141.64	(1.19)	13.05	(0.43)	0.01	0.931		
18	0.499	(0.011)	0.639	(0.010)	0.705	(0.006)	-160.32	(5.77)	20.11	(1.03)	0.22	0.639		
20	0.562	(0.012)	0.702	(0.010)	0.740	(0.006)	-204.21	(15.95)	22.43	(1.47)	1.76	0.185		
21	0.592	(0.009)	0.728	(0.010)	0.822	(0.006)	-164.71	(11.42)	11.32	(4.95)	1.86	0.173		
22	0.528	(0.010)	0.664	(0.011)	0.737	(0.006)	-165.46	(1.53)	18.76	(0.28)	0.02	0.896		
23	0.562	(0.011)	0.650	(0.012)	0.713	(0.005)	--	--	--	--	--	--		
25	0.451	(0.008)	0.540	(0.010)	0.560	(0.004)	--	--	--	--	--	--		
26	0.560	(0.010)	0.702	(0.012)	0.811	(0.007)	-143.45	(9.78)	0	(5.69)	2.14	0.144	x	
27	0.513	(0.010)	0.650	(0.011)	0.742	(0.006)	-140.90	(8.52)	12.60	(4.28)	0.58	0.445		

28	0.555	(0.008)	0.712	(0.010)	0.784	(0.006)	-159.58	(6.23)	13.58	(1.97)	0.41	0.520		
29	0.505	(0.009)	0.650	(0.010)	0.723	(0.006)	-152.02	(4.21)	17.31	(1.02)	0.15	0.698		
30	0.516	(0.011)	0.700	(0.013)	0.758	(0.006)	-143.34	(18.10)	11.21	(7.53)	4.25	0.039	*	
32	0.506	(0.008)	0.657	(0.009)	0.723	(0.006)	-154.56	(9.22)	17.73	(2.35)	0.85	0.356		
33	0.505	(0.009)	0.647	(0.009)	0.715	(0.006)	-158.93	(5.08)	19.19	(1.00)	0.22	0.641		
34	0.480	(0.010)	0.620	(0.011)	0.690	(0.006)	-149.08	(4.63)	19.23	(1.01)	0.13	0.722		
35	0.544	(0.010)	0.665	(0.011)	0.767	(0.006)	-151.65	(18.29)	13.61	(7.04)	3.81	0.051		
36	0.557	(0.023)	0.728	(0.014)	0.803	(0.007)	-156.43	(10.56)	10.61	(4.91)	0.42	0.517		
39	0.482	(0.008)	0.654	(0.008)	0.723	(0.006)	-128.81	(15.13)	8.93	(7.75)	4.26	0.039	*	
40	0.485	(0.010)	0.647	(0.011)	0.727	(0.006)	-125.38	(7.20)	6.02	(5.67)	0.72	0.396		
41	0.525	(0.010)	0.658	(0.011)	0.743	(0.006)	-154.99	(8.68)	16.42	(2.33)	0.54	0.464		
42	0.514	(0.011)	0.648	(0.010)	0.747	(0.007)	-135.11	(11.28)	9.66	(6.46)	1.29	0.256		
43	0.521	(0.011)	0.693	(0.012)	0.768	(0.007)	-138.04	(9.40)	7.14	(5.83)	1.07	0.301		
44	0.509	(0.012)	0.670	(0.013)	0.761	(0.007)	-130.28	(3.38)	0	(3.79)	0.15	0.695	x	
45	0.584	(0.011)	0.713	(0.012)	0.806	(0.006)	-166.29	(14.39)	13.31	(5.13)	1.94	0.163		
46	0.532	(0.011)	0.713	(0.012)	0.810	(0.007)	-142.22	(15.32)	0	(6.85)	4.83	0.028	*	
47	0.497	(0.010)	0.597	(0.010)	0.658	(0.006)	-190.89	(11.48)	26.18	(1.01)	0.95	0.331		
48	0.565	(0.010)	0.734	(0.010)	0.806	(0.006)	-156.01	(9.46)	10.00	(4.88)	1.07	0.300		
49	0.546	(0.009)	0.719	(0.009)	0.791	(0.007)	-147.51	(11.30)	8.15	(6.14)	2.05	0.152		
50	0.617	(0.012)	0.706	(0.011)	0.817	(0.007)	-180.77	(33.66)	15.47	(7.86)	13.30	$3 \cdot 10^{-4}$	*	
51	0.522	(0.012)	0.696	(0.013)	0.773	(0.007)	-134.84	(8.34)	2.58	(5.61)	1.10	0.294	x	

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52	0.511	(0.009)	0.658	(0.010)	0.726	(0.006)	-156.57	(7.19)	17.91	(1.68)	0.46	0.497		
54	0.545	(0.011)	0.693	(0.011)	0.770	(0.006)	-157.83	(0.83)	14.54	(0.22)	0.01	0.943		
55	0.514	(0.011)	0.625	(0.011)	0.697	(0.006)	-176.75	(12.34)	22.83	(1.57)	0.99	0.321		
56	0.519	(0.01)	0.659	(0.012)	0.754	(0.006)	-139.47	(8.17)	10.63	(4.94)	0.64	0.422		
57	0.530	(0.009)	0.661	(0.009)	0.732	(0.006)	-169.27	(3.30)	19.64	(0.55)	0.10	0.750		
58	0.555	(0.01)	0.723	(0.012)	0.759	(0.006)	-182.49	(23.94)	19.22	(4.92)	5.87	0.015	*	
59	0.483	(0.012)	0.633	(0.011)	0.687	(0.006)	-161.56	(16.85)	21.43	(3.44)	1.61	0.205		
60	0.547	(0.01)	0.697	(0.011)	0.770	(0.006)	-160.77	(3.55)	15.17	(0.88)	0.11	0.740		
61	0.506	(0.011)	0.659	(0.011)	0.744	(0.006)	-134.52	(2.20)	9.40	(1.34)	0.03	0.866		
62	0.551	(0.011)	0.715	(0.011)	0.786	(0.008)	-156.53	(10.18)	12.47	(4.32)	0.72	0.395		
63	0.514	(0.008)	0.649	(0.008)	0.723	(0.006)	-160.43	(1.99)	18.92	(0.39)	0.04	0.846		
64	0.498	(0.011)	0.648	(0.011)	0.728	(0.006)	-139.51	(3.68)	13.54	(1.37)	0.07	0.790		
65	0.579	(0.01)	0.699	(0.011)	0.776	(0.006)	-184.57	(11.24)	18.66	(1.74)	1.23	0.268		
66	0.511	(0.011)	0.666	(0.011)	0.725	(0.006)	-161.23	(14.65)	18.76	(3.54)	1.42	0.234		
67	0.591	(0.014)	0.659	(0.011)	0.770	(0.007)	-179.36	(45.29)	18.78	(9.13)	16.75	$4 \cdot 10^{-5}$	*	
68	0.482	(0.011)	0.625	(0.011)	0.689	(0.006)	-155.68	(9.73)	20.47	(1.93)	0.56	0.455		
70	0.557	(0.013)	0.650	(0.012)	0.744	(0.006)	-176.52	(29.17)	19.98	(6.42)	5.75	0.017	*	
71	0.538	(0.01)	0.708	(0.010)	0.761	(0.007)	-163.73	(20.15)	16.24	(6.49)	4.26	0.039	*	
73	0.568	(0.013)	0.713	(0.011)	0.771	(0.012)	-181.86	(8.13)	18.38	(1.36)	0.28	0.594		
74	0.504	--	0.720	(0.015)	0.746	(0.016)	--	--	--	--	--	--		
75	0.503	(0.034)	0.703	(0.029)	0.687	(0.026)	-197.57	(74.58)	23.87	(10.9)	4.43	0.035	*	

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