Supporting Information:

"Dipolar Couplings in Multiple Alignments suggest α helical Motion in Ubiquitin"
J. Meiler, W. Peti, and C. Griesinger

o) Experimental Data

Residual dipolar couplings (Hz)		DMPC/DHPC	DMPC/DHPC/SDS	purple mambrane fragments	CHAPSO/DLPC/SDS	CHAPSO/DLPC	CHAPSO/DPLC/CTAB 4%	CHAPSO/DPLC/CTAB 5%	polyacrylamide	Helfrich phase	Pf-1 phages	n-dodecyl-penta(ethylene glycol)
23	Ile	9.1	3.8	-17.1	---	-3.9	2.8	15.5	-0.9	29.3	20.0	-2.5
25	Asn	2.9	-2.6	-19.7	17.4	-1.7	4.3	21.2	0.3	15.7	12.4	-2.8
27	Lys	8.2	5.1	-15.0	8.6	-4.3	-2.0	11.8	-0.2	19.2	21.8	-4.3
29	Lys	7.2	2.7	-15.4	11.9	-2.0	---	15.2	-1.4	13.2	10.2	-3.9
30	Ile	8.7	3.6	---	9.3	-4.7	0.90	15.7	-0.6	21.9	19.0	-5.0
33	Lys	8.3	3.4	-19.3	---	-3.2	-0.4	15.4	-1.5	13.9	11.7	-3.9
D_{zz} (Hz)		-18.7	-30.5	33.9	-29.6	11.5	27.3	-57.8	5.35	-41.8	-26.4	12.1
Rhombicity		0.16	0.50	0.09	0.39	0.24	0.62	0.33	0.51	0.22	0.50	0.30
Euler-R(α°)		58	-88	54	85	78	-88	81	74	-46	41	-84
Euler-R(β°)		-57	-63	8	18	-61	-63	20	-67	25	12	-68
Euler-R(γ°)		67	72	62	56	67	72	83	71	73	11	83

For the complete set of dipolar couplings for all residues of Ubiquitin see table e) and J.-C. Hus, W. Peti, C. Griesinger, and R. Brüschweiler*, "Self-Consistency Analysis of Dipolar Couplings in Multiple Alignments of Ubiquitin", J. Am. Chem. Soc., ASAP Article 10.1021/ja029719s S0002-7863(02)09719-6, Web Release Date: April 19, 2003

oo) Complete Results: Fitted Values (min/max)

i	θ^i_{eff} [°]	φ^i_{eff} [°]	ϕ^i_{rdc} [°]	$\langle Y^i_{20} \rangle$ [1]	$\langle Y^i_{22} \rangle$ [1]	S^i_{rdc} [1]	η^i_{rdc} [1]	a^i [°]	b^i [°]
23	85	22	-205	0.60 (0.57/0.63)	0.09 (0.07/0.13)	0.97 (0.92/1.00)	0.21 (0.15/0.30)	19 (16/21)	0* (0*/0*)
25	95	-9	-177	0.57 (0.53/0.63)	0.02 (0.01/0.05)	0.91 (0.84/1.00)	0.04 (0.02/0.13)	17 (9/19)	7 (0*/15)
27	101	20	-205	0.53 (0.50/0.56)	0.01 (0.01/0.04)	0.84 (0.79/0.89)	0.02 (0.02/0.11)	19 (21/21)	16 (0*/19)
29	88	-5	-190	0.58 (0.53/0.62)	0.07 (0.03/0.09)	0.93 (0.84/1.00)	0.16 (0.07/0.24)	20 (15/23)	0* (0*/ 8)
30	94	11	-198	0.59 (0.55/0.63)	0.06 (0.03/0.11)	0.94 (0.87/1.00)	0.14 (0.07/0.26)	18 (15/22)	0* (0*/ 4)
33	84	-5	-169	0.58 (0.53/0.63)	0.01 (0.01/0.06)	0.93 (0.84/1.00)	0.03 (0.01/0.15)	15 (11/18)	6 (0*/16)
aver	91	6	-191	0.58 (0.54/0.62)	0.04 (0.02/0.08)	0.92 (0.85/0.99)	0.10 (0.05/0.20)	19 (16/21)	0* (0*/12)

*: no *b* value is found for these models

a) Significance of obtained asymmetries

To prove that the obtained asymmetries are significant and are smaller than the experimental error the following computer experiments were performed: (i) The spherical harmonics extracted from the set of dipolar couplings were used to back-compute the dipolar couplings; (ii) a second set of dipolar couplings was obtained by replacing the asymmetric part of the spherical harmonics (Y_{22} in C") with the average value found for the complete helix; (iii) the asymmetry was inverted by rotating the spherical harmonics in the coordinate system C" by 90° around the z''-axis. From the thus modified spherical harmonics a third set of dipolar couplings was back-computed.
The standard deviation between experimental and back-computed values for the first set lies with 0.7Hz in the range of the experimental error as discussed in reference 5. None of the 62 experimental values showed a deviation larger than 3Hz. If now the asymmetry is assumed as it is found for the complete helix the standard deviation increases slightly to become 1.3Hz and 2 data points have deviations larger than 3Hz. However, if the 90° rotated asymmetry is assumed the standard deviation jumps to 3.1Hz and 30 of the 62 data points have a deviation above four times the standard deviation of the method. The probability that this is found by chance is negligible which means in turn that the found common asymmetries for the helix N-H^N vectors is a real observation and cannot be an artifact due to measurement errors.

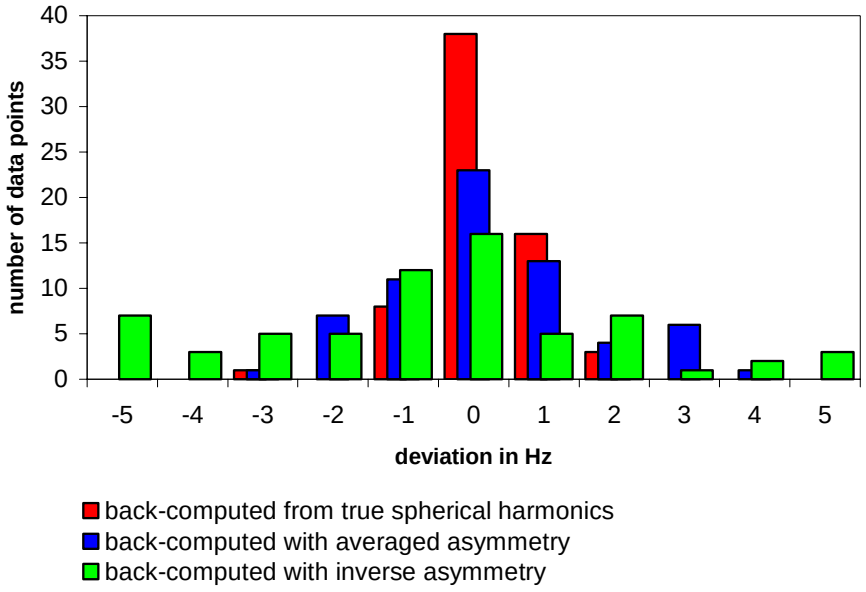


Figure S1: Distribution of the deviations of experimental and back-calculated dipolar couplings for the three scenarios: direct back-calculation assuming the found spherical harmonics (red), back-calculation with a common averaged asymmetry (blue), and with an inverted asymmetry (green).

b) Equations for finding parameters *a* and *b* from a diffusion in an asymmetric cone model.

I) $$r_e = \arcsin b / \sqrt{\cos(\nu)}\sqrt{b^2/a^2 + \tan(\nu)}$$

II) $$\langle Y_{20} \rangle = \int_{-\nu}^{\nu} \int_0^{r_e} Y_{20}(\mu,\nu) \bullet \sin\mu (d\mu)(d\nu)$$

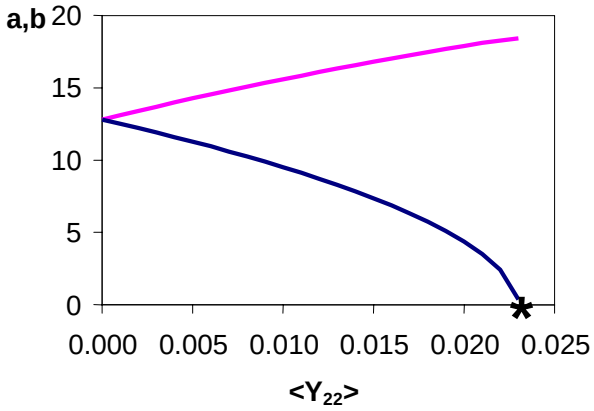
III) $$\langle Y_{22} \rangle = \int_{-\nu}^{\nu} \int_0^{r_e} Y_{22}(\mu,\nu) \bullet \sin\mu (d\mu)(d\nu)$$

IV) $$S^2_{rdc} = \frac{4}{5\pi}\left[\langle Y_{20} \rangle^2 + 2 \bullet \langle Y_{22} \rangle^2\right]$$

V) $$\eta^2_{rdc} = 2 \bullet \langle Y_{22} \rangle^2 \Big/ \left[\langle Y_{20} \rangle^2 + 2 \bullet \langle Y_{22} \rangle^2\right]$$

Eq. I describes the radius $r_{ellipse}$ of a point on an ellipse with the principle axes *a* and *b* in dependence of the phase ν. Using this radius the time averaged spherical harmonics $\langle Y_{20} \rangle$ and $\langle Y_{22} \rangle$ and subsequently the order parameter S^2_{rdc} and the asymmetry η^2_{rdc} can be expressed if a distribution of the vector in an ellipse is assumed (Eq. II-V). Experimentally obtained $\langle Y_{20} \rangle$ and $\langle Y_{22} \rangle$ allow to find parameters *a* and *b* by applying a least square fit protocol. However, it is not possible to find for every combination of $\langle Y_{20} \rangle$ and $\langle Y_{22} \rangle$ a pair of *a* and *b* as demonstrated in Figure S2. For a given $\langle Y_{20} \rangle$ *a* and *b* can be fitted up to a defined value of $\langle Y_{22} \rangle$. Of course alternative motional models exist that may fulfill such combinations of $\langle Y_{20} \rangle$ and $\langle Y_{22} \rangle$. For some combinations of $\langle Y_{20} \rangle$ and $\langle Y_{22} \rangle$ in the range of possible values we obtained the fit becomes also impossible. In Table 1 we marked those data points with *.

Figure S2: Dependence of *a* and *b* on $\langle Y_{22} \rangle$ for a constant $\langle Y_{20} \rangle = 0.575$.



c) Definition of the coordinate systems C, C', and C"

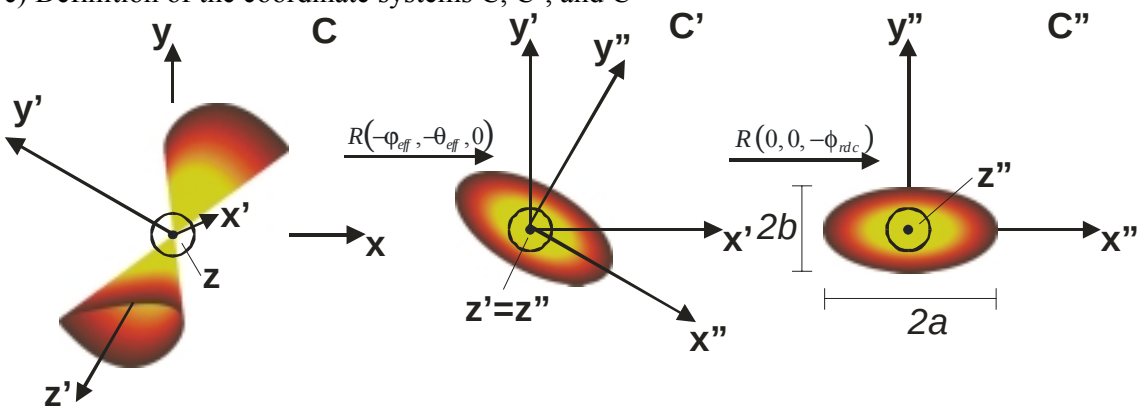


Figure S3: Procedure of the applied analysis. Leaving the molecular frame C the molecule is rotated firstly into the coordinate system C' by applying the rotation $R\left(-\varphi_{eff},-\theta_{eff},0\right)$ that puts the principal axis of the cone on the z-axis of the new coordinate system. A second rotation $R\left(0,0,-\phi_{rdc}\right)$ is applied to obtain the large axis of the ellipse parallel to the x"-axis of the final coordinate system C".

d) Computational analysis

All structures were obtained using the following computational setup: First a molecular dynamic simulation was applied using simulated annealing protocol (6500 steps a 5fs at 300K, 5000 steps a 5fs linearly decreasing the temperature to 200K, and 2000 steps a 5fs linearly decreasing the temperature to 100K). Afterwards the energy of the resulting structure was minimized (200 steps POWELL minimization). Both calculations were performed using the program X-PLOR[1] with the CHARMM22 force field[2,3] in vacuum. For every experiment 20 structures were computed and subsequent analysis was performed for the five structures with lowest energy. The experimental setup was optimized to address the question whether NOESY spectra would have been sensitive for such motions and to analyze the relative energies of the structures qualitatively. Correct absolute energies are not obtainable by this setup since NOE restraints influence the energy even if one subtracts their contribution to the overall obtained value. Moreover a water box would have been to be applied and more detailed analysis of local minima would have been necessary.

(1) Brunger, A. T. In;; Yale University Press: New Haven, CT, 1992.
(2) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comp. Chem.* **1983**, *4*, 187-217.
(3) MacKerell Jr., A. D.; Brooks, B. R.; Brooks, C. L.; Nilsson, L.; Roux, B.; Won, Y.; Karplus, M. In *The Encyclopedia of Computational Chemistry*; al., P. v. R. S. e., Ed.; John Wiley & Sons: Chichester, 1998; Vol. 1, pp 271-277.

e) Complete experimental data for Ubiquitin in Pf-1 phages (not yet published elsewhere)

ID	AA	RDC(Hz)	ID	AA	RDC(Hz)	ID	AA	RDC(Hz)	ID	AA	RDC(Hz)
1	Met	---	20	Ser	-2.19	39	Asp	-15.05	58	Asp	-5.30
2	Gln	6.97	21	Asp	11.10	40	Gln	-10.95	59	Tyr	-7.30
3	Ile	---	22	Thr	-22.88	41	Gln	-15.94	60	Asn	---
4	Phe	-2.54	23	Ile	19.98	42	Arg	---	61	Ile	-4.25
5	Val	5.48	24	Glu	---	43	Leu	18.71	62	Gln	-21.87
6	Lys	16.43	25	Asn	12.38	44	Ile	21.91	63	Lys	3.33
7	Thr	---	26	Val	---	45	Phe	22.57	64	Glu	-18.51
8	Leu	0.91	27	Lys	21.76	46	Ala	---	65	Ser	---
9	Thr	-17.50	28	Ala	18.19	47	Gly	14.60	66	Thr	-12.49
10	Gly	17.61	29	Lys	10.16	48	Lys	7.59	67	Leu	-2.83
11	Lys	12.81	30	Ile	19.02	49	Gln	5.36	68	His	19.27
12	Thr	18.57	31	Gln	---	50	Leu	17.29	69	Leu	---
13	Ile	8.22	32	Asp	15.08	51	Glu	7.26	70	Val	6.31
14	Thr	7.58	33	Lys	11.66	52	Asp	-0.94	71	Leu	-5.85
15	Leu	-10.54	34	Glu	---	53	Gly	---	72	Arg	---
16	Glu	-5.04	35	Gly	-7.59	54	Arg	1.16	73	Leu	---
17	Val	1.42	36	Ile	6.86	55	Thr	10.93	74	Arg	-4.15
18	Glu	10.28	37	Pro	---	56	Leu	-14.48	75	Gly	-3.65
19	Pro	---	38	Pro	---	57	Ser	---	76	Gly	-3.43