

Supporting Information

Magnetic susceptibility induced alignment of proteins in reverse micelles

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Complete Reference 4

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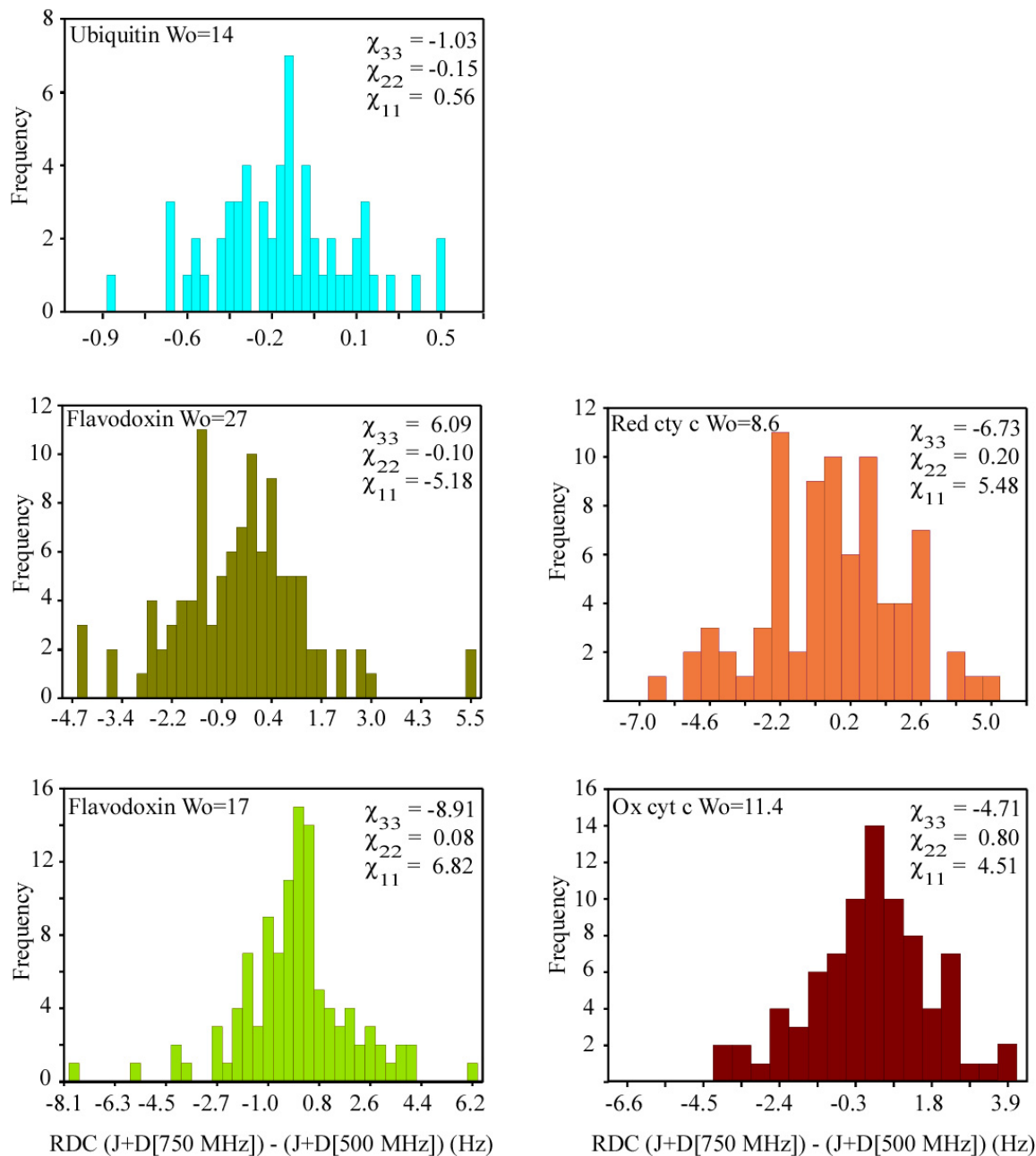


Figure S1. Histograms of the RDC values for the proteins in reverse micelles. The values of the principle axis system of the magnetic susceptibility tensor are given in the panels. The units are in 10^{-27}JT^{-2} . The values are determined from the histogram distributions with an additional 10% added to χ_{33} and χ_{11} edges to account for error in the measurement of these discontinuities.

Table S1. Residual dipolar couplings, $(^1J+D)^{(17.6T)} - (^1J+D)^{(11.7T)}$ for oxidized and reduced horse cytochrome c¹ encapsulated in reverse micelles.²

	ox cyt c		red cyt c		
Residue ³	RDC	RDC	Residue	RDC	RDC
D2	0.88	-2.51	A43	-1.86	2.32
V3	1.22	-4.19	G45	2.24	-2.69
G4			F46	-0.46	-1.19
K5			T47	0.65	-1.25
G6	1.74		Y48	-0.01	4.98
K7	3.06	-0.91	T49	2.19	
K8	0.37	4.3	D50	-0.64	-2.30
I9	2.33	-6.12	A51		0.83
F10	-2.00	-4.79	N52	0.12	2.02
V11	1.17	-1.75	K53	1.05	2.10
Q12	0.31	-2.94	N54	-0.55	-0.63
K13	-1.12	2.32	K55	0.63	3.54
C14		2.16	G56	-3.15	
A15	-0.18	-0.88	I57	-0.12	
Q16	0.60	0.07	T58	-0.62	-0.51
C17			W59	1.00	-0.41
H18	-3.65		K60		
T19	2.24	0.29	G61	0.53	-1.57
V20	-3.70		E62	-0.70	0.25
E21	0.80	-2.41	T63	0.30	-2.53
K22	0.32	0.22	L64	1.28	-1.04
G23	0.29	-1.26	M65	-1.52	-0.54
G24	1.97	0.40	E66	1.89	-2.31
K25	0.43	1.23	L68	2.43	-0.36
N26	2.39	-0.10	E69	3.91	0.17
K27	-1.64	-2.65	N70	0.96	-1.02
T28	3.00		K72	0.84	1.86
G29	-4.06		K73	-1.58	-1.02
N31	0.17	-2.89	Y74	0.00	0.69
L32	-2.43	1.84	I75	-2.54	-3.19
N33		-4.76	G77		0.33
G34			T78	1.97	0.71
L35	-1.67	-3.72	K79	0.44	
F36	-0.77	-2.46	M80		-4.66
G37	-0.73	-4.26	A81	4.11	1.28
R38	-1.67	-1.64	F82	-1.55	0.30
K39		1.64	A83	-2.46	-0.86
T40	-0.87	-0.32	I85		
G41	0.20	0.55	K86	-0.65	-1.21
Q42	2.12		K87		-1.33

K88	-0.26	-5.39	Y97	-1.88	-2.70
T89	-1.20	1.56	L98	0.82	1.36
E90	1.17	-5.65	K99	-1.11	0.37
R91	0.14	2.02	K100	1.35	2.53
E92	0.04	-1.09	A101	-2.38	-2.64
D93	0.08	-1.07	T102		-0.50
I95	-4.28	3.34	N103	-0.29	-2.68
A96	-0.18		E104	-0.35	-0.49

¹Recombinant horse ¹⁵N cytochrome c was prepared according to Rumbley, J. N.; Hoang, L.; Englander, S. W. *Biochemistry* **2002**, *41*, 13894-13901. and Liu, W.; Rumbley, J.; Englander, S. W.; Wand, A. J. *Protein Sci.* **2003**, *12*, 2104-2108.

²Oxidized cytochrome c reverse micelles were prepared by dissolving 2 mg of lyophilized powder in 22 μ l of buffer (50 mM KPhosphate, pH 5.8). The co-surfactants, sodium bis (2-ethylhexyl) sulfosuccinate (AOT), dodecyltrimethylammonium bromide (DTAB) and dodecyl tetraethylene glycol ether (C12E4) are dissolved in 800 μ l d₁₂ pentane in a ratio of 25:5:70 at 100 mM total concentration. The 20 μ l of aqueous protein solution is injected into the pentane / lipid solution and vortexed until clear. The molar ration of water to surfactant, water loading or W₀, was 11.4 H₂O/lipid as measured directly by ¹H NMR. The reduced cytochrome c is made in the same way, with the only difference in the buffer (50 mM KPhosphate, 50 mM NaAscorbate, pH 5.8). The water loading was measured to be 8.6.

³The ¹⁵N-¹H resonance assignments for the oxidized and reduced horse cytochrome c in reverse micelles were mapped from the assignments in the aqueous solutions of the proteins in the same buffers. The assignments are from Liu, W.; Rumbley, J.; Englander, S. W.; Wand, A. J. *Protein Sci.* **2003**, *12*, 2104-2108.

Table S2. Residual dipolar couplings, $(^1\text{J}+\text{D})^{(17.6\text{T})} - (^1\text{J}+\text{D})^{(11.7\text{T})}$ for oxidized flavodoxin⁴ encapsulated at water loadings of 17 and 27.⁵

Residue ⁶	flv W ₀ =17	flv W ₀ =27	Residue	flv W ₀ =17	flv W ₀ =27
	RDC	RDC		RDC	RDC
K3		0.31	E72	3.09	
I4	-2.14	-2.36	D74	-2.24	0.93
G5		-0.34	D75	-8.13	-4.64
L6	-1.05	-2.66	V76	1.76	0.98
F7	1.31	-0.38	D77	-2.15	-0.51
G9	0.47		F78	1.87	-1.35
E16	-1.21	0.51	G80	-0.30	0.55
S17	-1.28	-0.32	K81		-0.13
V20	-1.25	0.16	L82	-0.61	-0.77
A19	-0.69	-0.82	A84	-0.96	-0.87
I21		-1.49	F86	-0.28	-1.87
I22	0.09	1.98	G87	-1.96	1.62
R23		-4.71	G89	3.57	0.20
D24		2.49	Q91	-0.55	
F26	-0.44	-1.24	I92		-1.45
V30	0.26		G93	-0.95	-2.77
V31		-3.78	Y94	-0.73	
D35	-0.52	-0.15	A95	-1.10	0.08
V36	6.22	-1.74	D96	-1.98	-0.98
S37	-0.04	1.26	N97	0.37	0.57
Q38	0.20		F98	-1.96	-1.10
A39	0.45	1.16	Q99		-0.01
E40	0.34	-0.93	D100	-4.48	2.81
V41	-0.44	-0.19	A101	0.55	-0.32
T42	-0.36	-0.06	T102	-1.84	-1.61
D43	-0.18	-0.40	G103	2.34	-0.04
L44		-2.18	I104	-2.69	-1.38
N45	0.22	-2.12	E106		0.25
D46		-1.99	E107	0.16	
Y47	0.80	0.11	K108	-3.99	-1.57
Y49	-1.16	0.67	S110	-0.02	0.76
L50	1.95		Q111	1.00	-2.83
I51	0.84	-1.59	G113	1.71	-0.27
I52		-0.51	G114	-1.44	1.08
A54	-0.11	0.24	K115	1.42	-0.76
I59	-1.10	-1.47	V117	-0.23	-0.25
G60	-5.69	-2.71	G118	-0.71	0.23
E61	4.05		Y119	-1.48	2.63
L62	0.51		W120	-0.41	-0.42
S71		-1.58	S121		-4.69

T122	-0.31	0.88	E145	1.59	
D123	3.40	-2.79	D146	0.37	0.31
G124	0.06	-1.42	N147	0.43	-2.55
Y125	1.02	-0.50	Q148	2.51	2.17
D126	0.66	0.33	S149	-1.06	-1.75
F127	0.00	-0.16	D150	0.02	-0.78
N128	-0.12		L151	2.65	0.69
D129	1.00	-1.42	T152		-1.75
S130	0.28	-0.19	D153		-1.26
K131	0.77	-0.83	R155		-0.40
A132		1.40	K157	-0.83	-1.42
L133	2.89	1.63	S158	-0.16	-2.11
R134	0.44	-3.03	W159	-2.76	0.28
G136	-0.02	-0.84	V160	-3.03	
K137	-2.01		A161	1.26	-1.00
F138	-3.02	-2.30	Q162	-1.34	0.99
V139	2.49	5.54	S165	-0.63	0.88
L141	-1.76	0.15	F167	-1.76	-3.93
L143	3.78	5.45	G168	2.10	0.86
D144	-0.16		L169	0.22	0.52

⁴Recombinant oxidized *Cyanobacterium anbaena* ¹⁵N flavodoxin was prepared according to Liu, W.; Flynn, P. F.; Fuentes, E. J.; Kranz, J. K.; McCormick, M.; Wand, A. J. *Biochemistry* **2001**, *40*, 14744-1453.

⁵Oxidized flavodoxin reverse micelles were prepared from 10 µl of 10 mM flavodoxin in 50 mM KPhosphate, pH 5.5. The surfactant, 100 mM lysododecylamineoxide (LDAO) is dissolved in d₁₂ pentane with 4% hexanol. The aqueous protein solution is injected into the dissolved surfactant pentane solution and vortexed until clear. Two separate samples were made with different water loadings, 16.7 and 27.0. The water loadings were determined from the integration of the ¹H NMR spectra.

⁶The ¹⁵N-¹H resonance assignments for the oxidized flavodoxin in reverse micelles at both water loadings were mapped from the assignments in the aqueous solution of the protein in the same buffer. The assignments are from Liu, W.; Flynn, P. F.; Fuentes, E. J.; Kranz, J. K.; McCormick, M.; Wand, A. J. *Biochemistry* **2001**, *40*, 14744-1453.

Table S3. Residual dipolar couplings, $(^1J+D)^{(17.6T)} - (^1J+D)^{(11.7T)}$ for encapsulated ubiquitin.⁷

Residue ⁸	RDC	Residue	RDC
Q2	0.03	A46	0.19
I3	-0.20	G47	-0.20
F4	-0.21	K48	-0.09
K6	-0.52	Q49	-0.14
L7	-0.27	L50	-0.36
K11	-0.22	E51	-0.15
T12	-0.15	D52	-0.45
L15	-0.36	R54	-0.41
E16	-0.06	T55	-0.39
V17	0.06	L56	0.38
E18	0.21	S57	-0.14
S20	0.15	D58	-0.44
D21	-0.16	Y59	0.52
I23	-0.58	N60	0.50
K27	-0.55	I61	-0.41
A28	-0.33	Q62	0.29
K29	0.04	K63	0.14
I30	-0.14	E64	-0.26
Q31	-0.67	S65	-0.94
D32	-0.13	T66	-0.20
K33	-0.33	I67	-0.32
E34	-0.33	H68	-0.67
Q35	0.17	L69	-0.28
I36	-0.06	V70	-0.02
Q40	0.11	L71	-0.16
Q41	0.19	L73	-0.41
L43	-0.20	R74	-0.67
I44	-0.08	G75	-0.09
F45	-0.54	G76	-0.10

⁷Lyophilized ¹⁵N ubiquitin was dissolved in 15 μ l of buffer (50 mM NaAcetate, pH 5.0), 75 mM AOT was dissolved in 750 ml d₁₂ pentane. The protein solution was injected into the lipid pentane solution, then vortexed until clear. The water loading was 14.0.

⁸The ¹⁵N-¹H resonance assignments for ubiquitin in reverse micelles were from Wand, A. J.; Ehrhardt, M. R.; Flynn, P. F. *Proc Natl Acad Sci USA* **1998**, *95*, 15299-15302.

Table S4. Summary of structural statistics for protein structures in reverse micelles calculated with CNS using the experimental RDCs.⁹

	Ubiquitin	cytochrome c (oxidized)	cytochrome c (reduced)	flavodoxin (W ₀ =17)	flavodoxin (W ₀ =27)
Deviations					
RDCs ¹ H- ¹⁵ N(Hz) ¹⁰	0.090±0.001	0.044±0.001	0.038±0.002	0.017±0.000	0.029±0.001
Distance restraints ¹¹	0.034±0.001	0.079±0.002	0.090±0.003	0.050±0.001	0.058±0.001
Bonds(Å)	0.003±0.000	0.007±0.000	0.008±0.000	0.005±0.000	0.006±0.000
Angles(°)	0.584±0.006	1.219±0.013	1.503±0.075	1.114±0.023	1.303±0.014
Impropers(°)	0.716±0.004	1.451±0.059	1.606±0.056	1.030±0.023	1.253±0.011
ω dihedral(°) ¹²	0.355±0.016	1.227±0.115	1.401±0.065	0.701±0.086	1.133±0.093
Structure Ensemble ¹³					
Backbone (Å)	0.80 ± 0.47	0.24 ± 0.10	0.38 ± 0.20	0.24 ± 0.07	0.34 ± 0.16
Heavy atoms (Å)	1.12 ± 0.34	0.69 ± 0.08	0.84 ± 0.16	0.46 ± 0.06	0.55 ± 0.14
Structure Quality ¹⁴					
% residues in core region	79.55±0.87	72.57±1.48	64.58±2.42	76.03±2.44	76.61±1.15
%residues in allowed region	16.83±0.58	26.06±1.40	31.13±2.75	18.36±2.30	19.19±1.63
%residues in generously allowed region	3.50±0.67	0.92±0.57	3.32±0.81	5.09±0.59	4.10±0.55
%residues in disallowed region	0.12±0.23	0.46±0.57	0.92±0.77	0.55±0.37	0.07±0.13

⁹ Structural statistics are from the ensemble average of ~25% of the 100 simulated annealing (SA) structures accepted based on agreement with the constraints lists.

¹⁰ The RDC constraints were the experimentally measured field dependent $\Delta(J+D)^{15}_N \cdot ^1H(750MHz-500MHz)$ values. The SA protocol used these constraints with a ± 0.2 Hz deviation from the experimental values. The accepted structures contained no more than 2 violations greater than 0.2 Hz.

¹¹ The distance constraints were generated by an extraction from the starting PDB files. All atom pairs within 5 Å were selected and a subset containing a random 15% of the NOEs were used. The constraints were applied with a ± 0.2 Å deviation from the calculated value. The accepted structures contained no more than 2% of NOEs violated by more than 0.28 Å.

¹² The ω dihedral angle of 0° for the peptide bond was applied as a constraint to maintain the planar peptide geometry. The constraints were applied with a $\pm 1.0^\circ$ deviation from the planar value.

¹³ Rms deviations are calculated with MOLMOL for the ensemble of 24 structures for ubiquitin, 26 structures for oxidized cytochrome c, 28 structures for reduced cytochrome c, 24 structures for flavodoxin with W₀=17 and 28 structures for flavodoxin with W₀=27.

¹⁴ Calculated with PROCHECK (Laskowski, R. A.; Moss, D. S.; Thornton, J. M. *J. Mol. Biol.* **1993**, *231*, 1049-1067).

Table S5. Summary of REDCAT¹⁵ and PALES¹⁶ analysis for lowest energy protein structures in reverse micelles calculated with CNS using the experimental RDCs.

	Ubiquitin	cytochrome c (oxidized)	cytochrome c (reduced)	flavodoxin (W ₀ =17)	flavodoxin (W ₀ =27)
Magnetic susceptibility principal axis elements (REDCAT) ¹⁷					
χ_{11}	-3.07×10^{-6}	2.15×10^{-5}	2.82×10^{-5}	3.07×10^{-5}	-2.14×10^{-5}
χ_{22}	-3.96×10^{-5}	1.93×10^{-4}	2.61×10^{-4}	3.32×10^{-4}	-2.53×10^{-4}
χ_{33}	4.27×10^{-5}	-2.14×10^{-4}	-2.89×10^{-4}	-3.63×10^{-4}	2.74×10^{-4}
Euler Angles (REDCAT) ¹⁸					
$\alpha(^{\circ})$	0.0	9.4	-68.9	-54.1	-4.6
$\beta(^{\circ})$	98.0	281.5	172.9	82.2	343.0
$\gamma(^{\circ})$	71.5	-43.4	9.8	92.7	48.6
D_r/D_a ¹⁹	0.57	0.53	0.54	0.55	0.56
Normalized scalar products ²⁰ between tensors from the predicted steric alignment (PALES) ²¹ and the experimental alignment (PALES).	0.456	0.266	0.125	0.345	0.397

¹⁵ REDCAT analysis was performed for the lowest energy structure from the ensemble of accepted structures from the SA calculations. The maximum ¹⁵N-¹H coupling was set to 21646.4 Hz for an N-H bond length of 1.04 Å.

¹⁶PALES analysis was performed for the lowest energy structure from the ensemble of accepted structures from the SA calculations. The maximum ¹⁵N-¹H coupling was set to 21523.1 Hz for an N-H bond length of 1.038 Å. (Zweckstetter, M.; Bax, A. *J. Am. Chem. Soc.* **2000**, *122*, 3791-3792).

¹⁷ The magnetic susceptibility principal axis elements reflect the magnitude of the degree of alignment of the assembly.

¹⁸The Euler angles relate the magnetic susceptibility principal axis tensor to the molecular axis coordinate system.

¹⁹The D_r/D_a is the rhombicity R used in the CNS calculations listed in Table 1 in the text. This is 2/3 of the η value determined in the REDCAT analysis.

²⁰The normalized scalar product is defined as $\langle A^1|A^2 \rangle / (\langle A^1|A^1 \rangle^{1/2} \langle A^2|A^2 \rangle^{1/2})$ where A_m^1 and A_m^2 are the constants of the symmetric Saupe order matrix for the alignment of the respective molecules. (Sass, J.; Cordier, F.; Hoffmann, A.; Cousin, A.; Omichinski, J.G.; Lowen, H.; Grzesiek, S. *J. Am. Chem. Soc.* **1999**, *121*, 2047-2055).

²¹The steric alignment was predicted using the lowest energy structures from the CNS calculations. The steric alignment was predicted as a wall with the concentration of bicelle set at 0.05 mg/ml.