

Supporting Information.

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(i) Data used in the analysis :

Table S1

	NH	C'N	$^{\alpha}\text{C C}'$	$\text{H}_{(i)}\text{C}^{\alpha}_{(i)}$	$\text{H}_{(i)}\text{C}^{\alpha}_{(i-1)}$	H C'
Meier et al ^{s1}						
1. Alcohol mixture	54	55	55	35	15	47
Meier et al ^{s1}						
2. Phages	55	54	56	37	22	52
Ulmer et al ^{s2}						
3. Alcohol mixture	49	52	55	0	0	0
4. Phages (100mM salt)	50	53	54	0	0	0
5. Bicelles	49	52	51	0	0	0
6. NCPG	48	51	54	0	0	0
7. PCPG	48	53	54	0	0	0

Alignment tensor parameters for the different alignment media (determined from $^1\text{D}_{\text{CC}'}$ couplings):

Table S2

	$A_a (10^{-4})$	$A_r (10^{-4})$	$\theta (^{\circ})$	$\phi (^{\circ})$	$\psi (^{\circ})$
Meier et al.					
Alcohol	7.21	2.57	131.9	78.0	164.1
Meier et al.					
Phages	-12.774	-6.305	-23.8	156.5	32.0
Ulmer et al.					
Alcohol	8.558	2.232	100.0	82.8	172.1
Phages	-13.447	-1.306	-78.9	88.6	-34.8
Bicelles	15.087	3.951	-87.3	121.6	-10.7
NCPG	-10.883	-4.640	106.0	102.3	122.5
PCPG	-9.984	-6.184	-54.1	74.0	54.9

Normalized scalar products of 7 Saupe matrices for alignment tensors induced by the media listed in table S1 were also calculated. These were calculated using the method proposed by Sass et al. (3) Values close to 1

correspond to strongly correlated tensors, while values close to 0 correspond to uncorrelated tensors. Given in table S3 are the results when all couplings were used, while the results given in table S4 result from using only the ${}^1D_{C^{\alpha}C}$ couplings:

Table S3 Normalized Scalar Products of Saupe matrices (all couplings)

1	2	3	4	5	6	7	
1.0000	-0.0852	0.9422	-0.6792	0.7605	0.0675	0.5847	1
	1.0000	-0.2161	0.1027	-0.6659	-0.0975	-0.7957	2
		1.0000	-0.5818	0.7983	0.2316	0.5744	3
			1.0000	-0.3781	0.6523	-0.6052	4
				1.0000	0.3107	0.8541	5
					1.0000	-0.1721	6
						1.0000	7

Table S4 Normalized Scalar Products of Saupe matrices (${}^1D_{C^{\alpha}C}$ only)

1	2	3	4	5	6	7	
1.0000	0.0882	0.9203	-0.7330	0.7694	0.0130	0.5795	1
	1.0000	-0.0524	-0.0085	-0.5009	-0.0738	-0.6897	2
		1.0000	-0.6149	0.8025	0.2201	0.5295	3
			1.0000	-0.4443	0.6280	-0.6207	4
				1.0000	0.2803	0.8374	5
					1.0000	-0.2101	6

As illustrated in these tables, two of the alignment tensors (1 and 3) are closely related, these both result from alignment of protein G in a lyotropic medium based on a mixture of *n*-dodecyl penta(ethylene glycol) and *n*-hexanol. Three of the 27 data sets therefore have a higher level of redundancy (the duplicated $^1D_{HN}$, $^1D_{C'N}$ and $^1D_{C\alpha C'}$ in this alignment medium). We have nevertheless retained these data to increase effective signal to noise and thereby improve determination of the motional dynamics.

Mean and standard deviation of 3D GAF amplitudes with respect to secondary structure:

Table S5

	$\langle\sigma_{\alpha}\rangle$	$\langle\sigma_{\beta}\rangle$	$\langle\sigma_{\gamma}\rangle$
Helix	3.3±6.0°	4.5±4.3°	11.6±3.2°
Beta Sheet	3.7±5.3°	7.7±6.5°	12.1±10.3°
All	5.3±7.2°	7.0±5.9°	13.3±9.4°

(ii) Effects of site-specific differences between the two sequences:

RDC data were derived from protein G domains with two slightly different sequences, defined by the following mutations : V11A, I12L, K24E, E29A,

A34V and V47E. The crystal structure (1igd.pdb) that was used to model the average conformation in this study has identical sequence to the molecule studied by Ulmer et al comprising 5 of the 7 different alignment media (3 to 7 in table S1). We have therefore compared the amplitudes of σ_α , σ_β and σ_γ , determined using these 15 RDC datasets alone and using the entire data set.

For the important β -sheet sites we find no detectable difference in the ($\sigma_\alpha, \sigma_\beta$ and σ_γ) amplitudes greater than 4° . ($0 \pm 5, 1 \pm 4, 21 \pm 4$) $^\circ$ for all data sets (1-7) compared to ($0 \pm 5, 0 \pm 4, 21 \pm 4$) $^\circ$ for data sets (3-7) for V11A. Similarly ($10 \pm 7, 13 \pm 4, 0 \pm 7$) $^\circ$ compared to ($11 \pm 7, 10 \pm 4, 4 \pm 7$) $^\circ$ for I12L. Idem for K24E: ($4 \pm 5, 0 \pm 4, 17 \pm 5$) $^\circ$ compared to ($0 \pm 5, 0 \pm 4, 19 \pm 5$) $^\circ$. E29A gives ($2 \pm 8, 4 \pm 5, 19 \pm 4$) $^\circ$ compared to ($0 \pm 9, 14 \pm 5, 14 \pm 5$) $^\circ$ and A34V gives ($0 \pm 6, 13 \pm 4, 9 \pm 7$) $^\circ$ compared to ($0 \pm 5, 12 \pm 4, 15 \pm 7$) $^\circ$. V47E gives ($12 \pm 4, 6 \pm 5, 10 \pm 5$) $^\circ$ compared to ($10 \pm 4, 0 \pm 5, 11 \pm 5$) $^\circ$.

In summary, the amplitudes are very similar for residues 11, 12, whether we use data from exactly the same sequence, or both datasets simultaneously. For residues 24, 34 and 47 the differences are small (within the estimated error bar). For residue 29 there is a slightly bigger difference for one of the three amplitudes (β).

In addition to these observations, when we fit dynamic amplitudes using

only data sets 3-7, we still get an improved reproduction of data sets 1-2 (using cross-validation of all couplings – vide infra) compared to the static model.

We are therefore satisfied that the two sequences are very similar both structurally and dynamically.

(iii) Cross Validation of the 3D GAF Dynamic Description.

Due to the large number of RDCs used in the fitting procedure, cross-validation techniques can be used to establish the validity of the proposed dynamic model. In order to test this, the analysis was repeated, each time in the absence of one of the data sets, either the $^1D_{HN}$ data, or removing all data from each individual alignment medium in turn. The experimental values of the removed couplings were then compared to those predicted using the resulting dynamic model derived in their absence. Figure S3 shows the comparison, for all of the ‘free’ RDCs (where ‘free’ refers to the data that are not used in the fitting procedure), of experimental $^1D_{HN}$ values compared to calculated values using the static model and the dynamic model (determined from the remaining RDCs). The slope of the line accompanying the data is equal to 1, and all tensors were normalised such that the maximum coupling was equal to 1. As expected if the dynamics are real, simulated values from the static model tend to overestimate the true

value whereas the dynamic values are in general in better agreement. In addition the total χ^2 for the 'free' RDCs calculated using the dynamic model is 96, while for the static model this is 184.

Figure S4 shows the site-specific difference $\Delta\chi^2$ ($\Delta\chi^2 = \chi^2_{\text{stat}} - \chi^2_{\text{dyn}}$; this should be positive if the dynamic model improves the fit), between the static and dynamic χ^2 for the 'free' $^1\text{D}_{\text{HN}}$ RDCs, (the figure shows the average ($\Delta\chi^2$) values calculated over the seven cross-validation runs for each NH data set). Where this value is positive, the 3D GAF dynamic description provides an improved fit to the 'free' data set. Only 10 sites have negligible average $\Delta\chi^2$ values, between -0.05 and +0.05, over the seven calculations, all coinciding with sites experiencing low amplitude motions. The remaining 43 sites have positive average $\Delta\chi^2$ values, ranging from 0.06 to 3.05 (25). There are therefore no sites where the data are reproduced less well by the dynamic model.

We have repeated this analysis using all data from individual alignment media. The results are summarised in figures S5 and S6, for the X-ray and RDC-refined structures. Again $\Delta\chi^2$ is positive for nearly all sites. Finally couplings from the most independent data set (aligned in negatively charge polyacrylamide gel) are also found to be significantly better predicted using the motional models determined from the remaining data (figure S6). Taken

together these results strongly support the validity of the proposed dynamic models determined in this study.

(iv) Effect of Variation of the NH Distance.

We have estimated the effect of uncertainty in the position of the amide proton, as this affects the geometric dependence of four of the treated couplings differently. Analysis of the direction of the NH vector with respect to the $^{\alpha}\text{C}$ - $^{\alpha}\text{C}$ axis in high resolution X-ray and neutron scattering protein structures reveals a well-defined value of $(76.1 \pm 0.02)^{\circ}$ for 900 peptide planes (4), suggesting that the effect of the NH distance is the most important unknown geometric factor. The entire analysis was repeated over a range of NH distances to determine the optimal value from the total target function, of the free, and work data sets. Figures S3a and S3b show the profile of the χ^2 with respect to distance for the analysis where the bicelle-aligned data were removed (the 'free' set in this case). A similarly located minimum is found for both 'work' and 'free' data sets. A minimum was found in target functions comprising both fitted and non-fitted data, at r_{NH} values of 1.02\AA . This value was used throughout the procedure, although variation of the parameter by $\pm 0.01\text{\AA}$ has negligible effect on the described profiles.

(v) Effects of Structural Differences on the Fitting of GAF Amplitudes.

Although the effects of structural noise when fitting motional amplitudes from RDC data have been analysed in detail (5), it is nevertheless instructive to compare the results of the 3D GAF analysis when a different crystal structure is used to describe the average conformation. We have therefore used an RDC-refined NMR structure (1pe7) (2) and two lower resolution X-ray crystallographic structures (1pga, 2.1Å and 1pgb, 1.9Å (6)), with 6 mutations (I11A, I12L, K24E, E29A, A34V and V47E) compared to 1igd to determine the effects of structural uncertainty on the extracted 3D GAF amplitudes. The method is shown to be remarkably robust in this respect, no doubt due to the large number of RDCs used in the analysis. The motional amplitudes from the RDC-refined structure are shown in figure S1, reproducing the values derived from 1ugd virtually identically. The S^2 values extracted from 1pgb and 1igd are compared in figure S2. The analysis with the lower resolution structures reproduces all of the observations of interest and their associated amplitudes, including the dynamic network in the β sheets, and the increased dynamics in the loop and strand $\beta 2$. The mean and standard deviation of differences in order parameters calculated from the extracted amplitudes from 1igd and 1pga and 1pgb are $\Delta S^2 = 0.06 \pm 0.06$ and 0.06 ± 0.07 respectively. The only significant differences are

associated with the three sites where the χ^2 of the fit is far worse (between 3 and 6 times larger) for the lower resolution structure. Otherwise the reproduction of the motional averaging is remarkably similar.

(vi) *Table of values of 3D GAF amplitudes and associated uncertainty (in units of degree).*

peptide plane (numbered using NH)	Alpha (°)	uncertainty	Beta (°)	uncertainty	Gamma (°)	uncertainty	Number of RDCs
7	0	8	0	6	36	11	13
8	1	4	0	4	0	6	23
9	0	5	0	4	22	4	25
10	0	5	13	3	0	6	23
11	0	5	1	4	21	4	25
12	10	7	13	4	0	7	23
13	16	4	14	3	7	6	25
14	25	3	8	5	2	5	24
15	1	5	7	4	30	4	25
16	0	6	23	3	15	6	25
17	7	5	8	4	33	5	24
18	13	5	12	5	23	2	25
19	12	6	0	4	26	3	26
20	11	6	9	5	17	7	25
21	11	7	0	4	22	3	26
22	4	5	0	4	18	6	24
23	3	5	6	5	12	4	26
24	4	5	0	4	17	5	25
25	7	5	14	4	7	5	25
26	8	5	8	5	0	5	26
27	12	5	7	4	16	5	26
28	0	4	1	3	11	6	25
29	2	8	4	5	19	4	25
31	0	4	1	4	11	5	24
33	0	5	0	3	9	5	25
34	0	6	13	4	9	7	23
35	0	5	5	4	11	6	17
36	0	5	6	4	11	6	22
37	12	6	1	4	10	5	25
38	0	6	9	5	11	6	25
39	0	5	3	4	18	6	25
40	7	8	10	5	10	6	23
41	18	4	0	4	11	5	23
42	9	5	6	5	17	4	22

43	0	5	11	4	17	3	23
44	12	7	0	4	17	3	23
45	31	4	15	4	0	6	24
46	18	6	13	5	32	3	26
47	12	4	6	5	10	5	27
48	0	5	11	3	1	8	24
49	0	5	18	2	5	5	26
50	0	5	5	4	17	6	22
51	0	5	17	3	8	5	27
52	0	5	10	4	4	7	24
53	0	8	0	5	25	3	27
54	11	7	5	4	10	6	22
55	0	4	10	5	17	3	27
56	0	5	11	3	0	6	25
57	0	5	15	2	0	4	23
58	0	5	0	3	24	4	25
59	0	6	16	3	0	6	26
60	0	5	6	4	21	4	25
61	0	5	1	4	18	7	22

(vii) Table of values of RDCs.

Data from reference (S2) are available on the web at the address :

http://pubs3.acs.org/acs/journals/supporting_information.page?in_codon=jacsat&in_volume=125&in_start_page=9179

Data for the two remaining media are given here :

RDCs - Medium 1

Peptide Plane	$^1D_{HN}$	$^2D_{HC'}$	$^2D_{HC}^{\alpha}$	$^2D_{HC}^{\alpha(i-1)}$	$^1D_{C'N}$	$^1D_{C}^{\alpha C'}$
7	12.4931	2.7521	-1.7663	3.8706	-2.7890	0.6211
8	20.9458	-3.1311	###	###	1.0420	-1.5704
9	22.2212	-1.5506	###	2.0126	-0.5895	1.5196
10	9.9892	-3.9311	###	###	1.0150	-0.6719
11	1.8838	-3.8566	5.6740	###	0.8405	5.0567
12	-1.5298	-1.3053	###	###	0.9060	-1.1719
13	-16.6138	-0.8842	6.5508	###	0.9105	4.1465
14	3.1582	-5.7574	###	-2.1767	1.5155	-1.5879
15	-9.8394	-1.4939	5.0286	###	0.8070	3.5665
16	-16.5639	3.4396	0.5701	###	-0.0600	-1.1992
17	4.8183	0.4880	###	1.4168	-0.7290	-0.0097
18	4.6640	1.0906	-3.7660	###	-0.1295	-3.3652
19	7.7417	-7.5981	###	-2.0211	-0.5120	-0.3046
20	6.7075	-2.1180	0.9108	0.4885	0.0100	1.2558
21	14.1801	2.3826	-2.6081	3.7843	-2.1145	0.2793
22	21.4384	-2.9518	-3.1630	###	-0.5455	0.0020
23	25.1015	-3.7972	-0.5740	3.1827	-1.1470	3.0586
24	23.3349	-1.8646	-3.3460	1.8952	-1.0230	-1.1328
25	10.7500	-6.5936	3.5508	###	1.6820	4.3203
26	13.7969	4.6403	-4.7209	4.1613	-2.9805	-1.9707
27	18.3130	-1.8261	-5.1445	###	0.1515	-3.1367
28	19.3081	-6.4045	0.6281	###	1.4900	2.0977
29	2.6641	5.0289	-5.3142	###	-2.9275	-1.7597
30	11.9502	-0.6264	-4.5278	###	###	-4.4980
31	19.3750	###	2.2746	###	-0.4735	5.1093
32	7.3413	-3.6926	0.1248	-1.6412	0.9250	-0.2930
33	-1.0371	6.2443	-5.1677	###	-2.2120	-3.9375
34	14.9712	-3.8255	###	###	0.0905	-1.0566
35	11.3422	###	###	###	1.7475	3.6562
36	-8.2751	6.1256	###	###	-1.8225	-1.2402
37	0.5141	1.8635	-4.9293	1.6087	0.0755	-4.3242
38	10.3194	-3.8638	3.1952	###	0.0910	4.1680
39	2.1841	-3.8107	2.5815	-2.2565	2.1055	1.6953
40	-7.6488	7.9844	###	2.3553	-2.6755	-2.0429
41	4.1753	0.3065	###	###	0.5515	-2.4882
42	13.1924	-6.0355	4.0818	###	1.0480	5.3496
43	-26.0717	5.7064	1.9268	###	-1.0555	0.1815
44	-10.4919	0.6982	###	###	1.0630	-3.2246
45	-20.9619	1.3735	3.4528	###	0.7895	1.9806

46	-22.2378	2.8143	1.0718	-2.4451	1.0895	-2.1094
47	-28.2772	2.7431	2.3834	-2.1989	1.4560	-0.7539
48	-12.2771	7.1727	###	2.1780	-2.3790	-1.0118
49	3.4121	1.5794	-4.1810	###	-0.0150	-4.8379
50	10.5562	-3.6597	###	###	0.7580	-0.4024
51	18.2402	-0.6827	-5.7355	-2.1399	-0.2790	-3.8926
52	9.5537	-1.0964	-0.2430	1.6320	-0.3595	0.8242
53	3.6298	0.6876	-0.3892	1.2742	-0.7840	0.4472
54	2.7650	-1.4974	###	###	1.1335	-1.4883
55	15.6665	3.5612	-5.3618	3.3438	-2.5435	-2.2968
56	23.6826	-3.8472	-3.6488	###	-0.4015	-1.8184
57	15.3400	-7.2868	###	###	2.3840	-0.7812
58	-1.0977	-2.4904	3.7165	###	0.5215	4.0937
59	-6.3373	-1.1752	-0.0523	-2.0982	1.4060	-0.7832
60	-13.6529	-1.2809	6.0987	###	0.8555	4.0860
61	-11.2298	2.8183	###	###	-0.0645	-1.3419

RDCs - Medium 2

Peptide Plane	$^1D_{HN}$	$^2D_{HC'}$	$^2D_{HC}^{\alpha}$	$^2D_{HC}^{\alpha(i-1)}$	$^1D_{C'N}$	$^1D_{C}^{\alpha C'}$
7	0.5942	2.0544	###	0.6774	-0.8800	-1.5820
8	-4.8621	0.1295	###	###	0.8300	-1.2501
9	-7.2084	-0.1333	0.6313	###	0.9500	-0.6406
10	-8.2958	1.9176	###	###	0.5450	-1.5352
11	-10.3233	1.0493	0.9057	###	0.4950	0.3008
12	-7.9105	1.7561	###	###	-0.6250	-0.8770
13	-10.4736	1.8660	0.0968	###	0.1000	-1.0253
14	-2.5974	2.3196	###	###	-0.6800	-1.9903
15	-7.6751	-0.4739	0.7250	###	0.8850	-0.3281
16	-5.8062	0.2267	2.1506	###	0.0000	1.6016
17	-6.9481	1.6001	###	###	0.4850	-2.0078
18	-8.6532	2.0354	0.6408	###	-0.4750	0.1075
19	-8.8226	1.6397	-0.3808	-1.3665	0.6050	-1.7636
20	-10.0250	-0.4449	0.8510	-2.2529	1.4400	-0.4298
21	-8.4549	2.3536	-0.0778	###	-0.1000	-0.7695
22	-6.8134	-0.6756	0.4526	###	1.1950	-0.4317
23	-4.7507	0.7459	0.1571	###	-0.0350	0.4219
24	-5.0370	0.4293	###	-1.7622	0.8700	-1.3593
25	-4.1052	1.0379	2.0740	###	-1.1100	2.2872
26	-4.6784	1.9712	-0.0299	1.1125	-0.6400	-0.1953
27	0.7197	-3.1076	1.6131	-1.6046	1.4800	0.7832
28	-0.9055	1.5661	-2.5991	-1.2992	0.1350	###
29	7.2657	0.7018	###	1.7872	-0.9500	-0.3827
30	8.5708	###	0.5489	###	1.2300	1.3692
31	1.9038	1.0860	-0.1353	###	-0.4850	0.9628
32	1.3945	2.4371	###	###	-0.7350	-2.7597
33	11.9017	-2.2840	-0.7860	###	0.4800	0.3730
34	6.1601	-2.3842	###	###	0.3950	1.9414
35	2.1153	2.4285	###	###	-0.5050	-1.4922
36	###	2.2938	###	###	-1.5450	-2.3750
37	13.4474	###	-0.1533	###	0.9850	0.5977
38	6.8349	-1.3642	0.5220	###	-0.4200	1.4453
39	2.4439	2.1679	###	###	-0.8800	-2.7911
40	9.5755	0.3094	###	1.3980	-0.9650	-0.5117
41	11.7067	###	0.6702	###	0.8300	1.6114
42	-1.2153	0.5955	0.3595	###	-0.4600	0.6523
43	0.3702	###	###	###	-0.9150	-2.2793
44	12.6320	###	-0.1698	###	0.8200	0.6875
45	6.7143	###	1.6033	###	0.8550	2.0059
46	3.6879	###	-0.0960	0.4233	-0.1200	0.6191
47	-5.8251	1.5963	0.8456	1.6041	-0.6350	-0.1562
48	-9.8089	2.0191	###	###	0.2750	-1.9884
49	-10.1718	1.8194	1.3294	0.1472	0.0000	0.4199
50	-8.5412	1.4269	###	###	0.1450	-2.2891
51	-7.9427	1.7706	0.6561	0.3070	-0.2800	0.7440
52	-4.5601	2.7091	###	###	-0.4700	-2.9609
53	9.7017	-0.9435	-0.9291	0.9205	-0.9050	0.5137
54	6.3050	###	1.6288	###	0.9900	1.8536
55	-7.9409	3.1105	1.0274	1.8209	-1.0650	0.6133
56	-6.4281	-0.8922	0.3402	###	1.3400	-0.8262
57	-5.7600	1.5732	###	###	-0.4550	-0.1290
58	-12.0563	0.5013	0.6116	###	1.3450	-1.7852

59	-10.8328	2.3094	0.2221	###	0.2400	-0.6738
60	-11.9871	1.6221	0.0913	###	0.6350	-2.0137
61	-10.4998	2.5154	###	###	0.2550	-1.2403

¹ See experimental methods on page S1.

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