Modulating protein alignment in a liquid crystalline medium through conservative mutagenesis

Lishan Yao and Ad Bax*

Laboratory of Chemical Physics, NIDDK, National Institutes of Health, Bethesda, MD 20892-0520

Table S1. GB3 dipolar couplings measured in PF1 for A, K19AD47K; B, K19ED40N; C, K19EK4A C-His-tag; D, K19EK4A N-His-tag; E, K19AT11K; F, K19EK4A. The estimated errors are A, 0.1 Hz; B, 0.08 Hz; C, 0.20 Hz; D, 0.18Hz; E, 0.07Hz; F, 0.07Hz.

	A	B	C	D	E	F
3	-9.27	-10.13	-4.10	-11.36	-11.66	-6.47
4	-6.01	-13.24	-5.86	-13.52	-8.72	-9.13
5	-6.01	-6.23	-1.44	-9.37	-0.75	-3.72
6	-5.47	-6.61	0.13	-10.80	-4.28	-3.73
7	-3.87	3.14	1.84	-6.72	2.33	-0.96
8	-8.56	2.32	8.19	-5.36	-4.28	3.68
9	-0.40	2.94	-0.50	-2.62	7.07	-1.28
10	-11.21	7.42	N/A*	-4.86	-7.00	5.52
11	-10.06	4.50	N/A*	0.15	-5.65	7.95
12	-5.69	0.20	0.26	-7.81	-2.27	-1.75
13	-4.54	-8.77	-1.64	-10.40	-4.67	-4.92
14	-6.18	-7.22	-1.86	-9.75	-3.30	-4.76
15	-5.97	-6.87	-1.08	-11.02	-3.63	-5.02
16	-5.55	-10.19	-3.70	-11.29	-4.59	-6.64
17	-6.19	-12.49	-4.99	-12.20	-8.76	-7.90
18	-3.86	-11.14	-6.66	-12.57	-11.37	-9.56
19	-4.77	-10.00	-5.65	-11.91	-11.30	-8.98
20	-3.35	-0.09	-1.42	-9.24	-10.33	-3.44
21	-5.74	-2.68	N/A*	-10.75	-11.95	-4.89
22	9.54	-10.44	-8.39	-5.59	-6.11	-10.30
23	7.21	-12.65	-7.39	-7.69	-6.40	-10.02
24	14.03	-6.10	-3.62	6.17	3.06	-3.70
25	19.38	-3.76	-7.68	3.99	4.45	-7.09
26	10.40	-11.02	-9.04	-4.49	-4.93	-10.49
27	9.77	-10.85	-5.58	-1.52	-2.84	-6.82
28	16.72	-3.48	-3.80	8.98	6.85	-1.48
29	16.57	-6.66	-8.84	1.15	0.78	-8.72
30	10.32	-10.98	-6.72	-2.10	-2.85	-8.68
31	9.85	-5.27	-0.51	7.26	2.12	0.14
32	19.20	-1.17	-5.34	12.20	9.26	-2.36
33	15.78	-7.48	-7.67	2.48	1.23	-7.76
34	9.13	-9.33	-3.78	0.14	-1.81	-5.32
35	12.16	-2.95	-0.88	10.49	5.20	1.03
36	18.94	-3.27	-6.53	8.44	6.79	-4.40
37	5.70	-12.55	-6.04	-5.73	-5.26	-9.42
38	-8.82	4.86	10.85	6.04	-3.23	11.77
39	3.87	4.26	2.99	16.45	10.49	8.02
40	-1.05	3.94	8.36	10.42	1.54	9.55
41	-0.80	5.39	8.68	10.52	4.35	9.09
42	-10.25	6.36	11.84	1.48	-5.60	10.33
43	-6.10	8.47	5.44	-6.58	-0.86	2.80
44	-5.96	-6.13	0.34	-10.51	-2.14	-3.37

45	-6.92	-6.84	-1.41	-8.87	-1.94	-4.48
46	-6.95	-11.46	-4.45	-12.40	-6.16	-6.65
47	-3.11	-2.01	N/A*	-3.93	4.36	-2.25
48	5.52	2.96	-1.05	11.35	13.21	2.73
49	10.79	7.57	-3.20	8.10	14.59	0.14

50	-5.03	-11.48	-4.07	-11.85	-5.50	-7.32
51	-7.94	-12.50	-5.32	-12.62	-9.09	-7.41
52	-6.26	-7.51	-3.28	-8.19	-0.91	-4.45
53	-6.31	-4.13	1.77	-9.29	-3.42	-1.82
54	-6.10	1.51	2.64	-8.55	0.20	-0.35
55	-7.18	-0.60	5.99	-7.18	-4.26	1.00
56	-5.31	6.15	4.23	-6.53	0.39	1.54

* The couplings are unavailable due to weak signal intensity.

Table S2. Normalized scalar products between the alignment tensors obtained for GB3 in 11 aligning orientations, derived using SVD fitting to the GB3 NMR structure 2OED. A', Pf1; B', Bicelle; C' and D', negatively and positively charged polyacrylamide gels; E', PEG; A, K19AD47K; B, K19ED40N; C, K19EK4A C-His-tag; D, K19EK4A N-His-tag; E, K19AT11K; F, K19EK4A (wild type GB3 was used in A', B', C', D' and E').

	B'	C'	D'	E'	A	B	C	D	E	F
A'	-0.393	0.631	-0.611	-0.588	0.544	0.492	-0.232	0.700	0.945	0.174
B'		0.336	0.856	0.802	-0.371	-0.344	-0.273	-0.930	-0.552	-0.627
C'			-0.140	0.245	-0.121	0.391	-0.030	-0.029	0.403	0.057
D'				0.582	-0.141	-0.655	-0.497	-0.886	-0.659	-0.838
E'					-0.831	-0.276	0.277	-0.875	-0.746	-0.127
A						0.037	-0.669	0.530	0.706	-0.381
B							0.508	0.438	0.441	0.641
C								0.081	-0.235	0.879
D									0.802	0.522
E										0.172

Table S3. Generalized scalar product between the experimental alignment Saupe matrix and the corresponding matrix predicted by PALES.[a]

	K19AD47K	K19ED40N	K19EK4A-C-his-tag	K19EK4A-N-his-tag	K19AT11K	K19EK4A
Dot product	0.891	0.509	0.482	0.943	0.169	0.591

[a] Low values for K19ED40N, K19EK4A-C-his-tag, and K19AT11K are attributed to the much weaker alignment observed and predicted for these mutants, which makes the predictions very sensitive to orientations of the disordered sidechains of charged residues (see Zweckstetter, M.; Hummer, G.; Bax, A., *Biophys. J.* **2004,** *86,* 3444-3460).

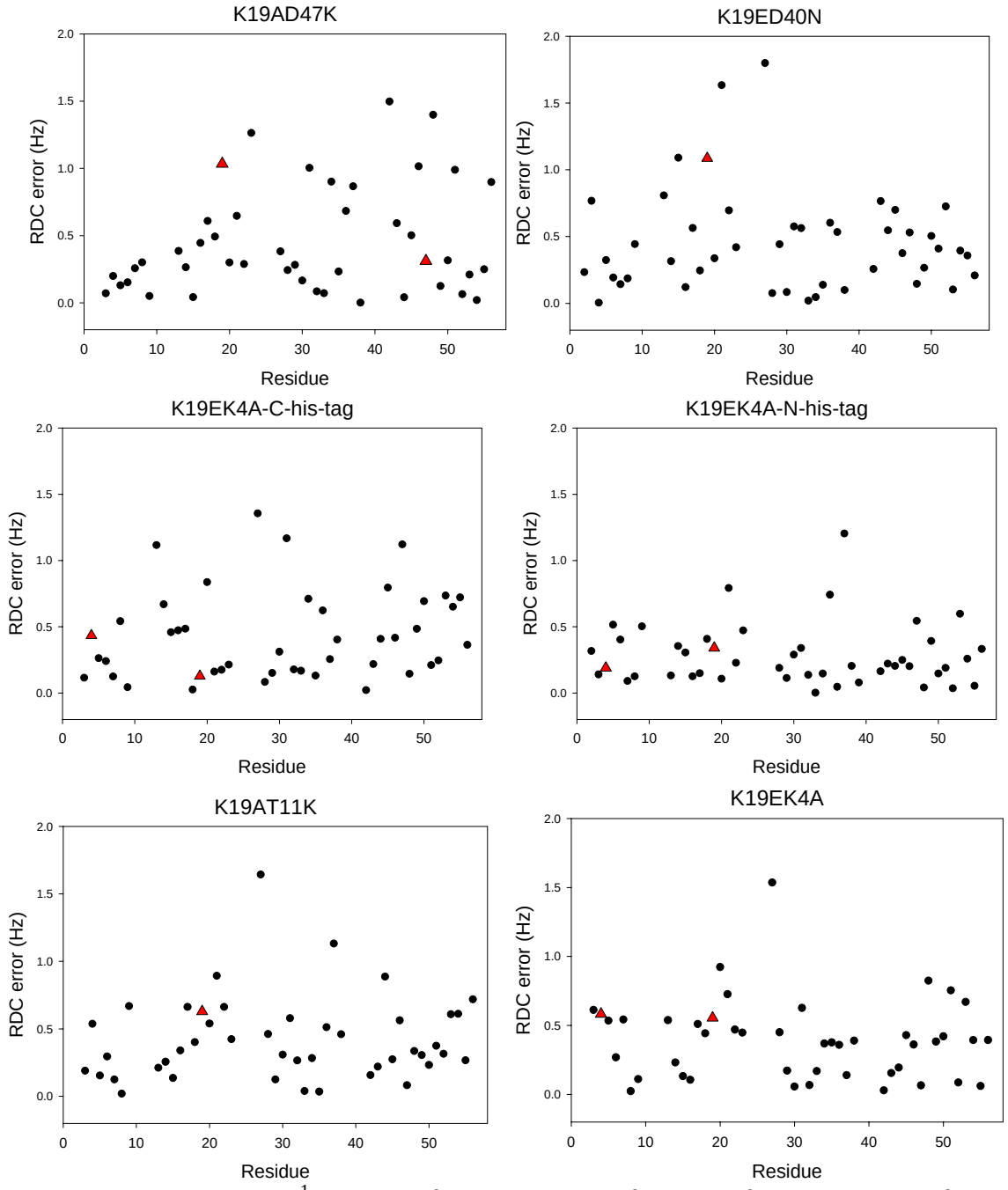

Figure S1. The backbone $^{1}D_{NH}$ RDC fitting errors as a function of residue number for the six different GB3 mutants. The error is defined as the absolute difference between the SVD-fitted and experimental RDCs. The mutated residues are marked as red triangles, with black dots for all other residues.