Multiple alignment of membrane proteins for measuring residual dipolar couplings using lanthanide ions bound to a small metal chelator

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Supporting Information

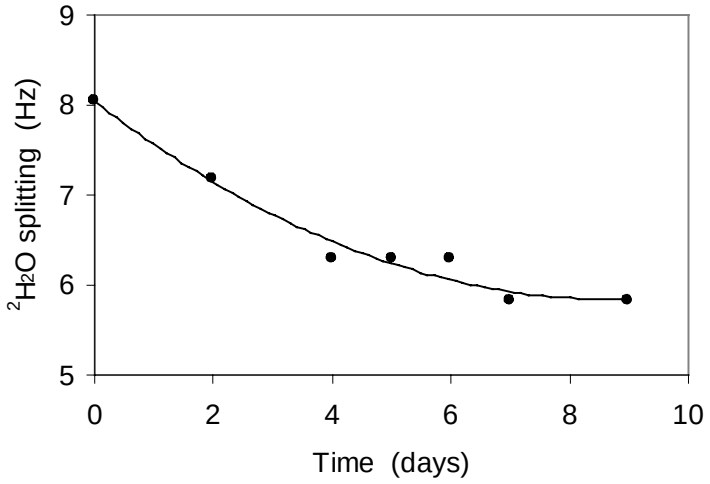

Figure S1. Decrease in deuterium splitting over time. Measurements were made at 47 º C on a Bruker DRX 600. Sample conditions were 6% polyacrylamide, 50 mM potassium phosphate, 5% LPPG, 1 mM subunit c, pH 6.8. The curve is a simple trendline.

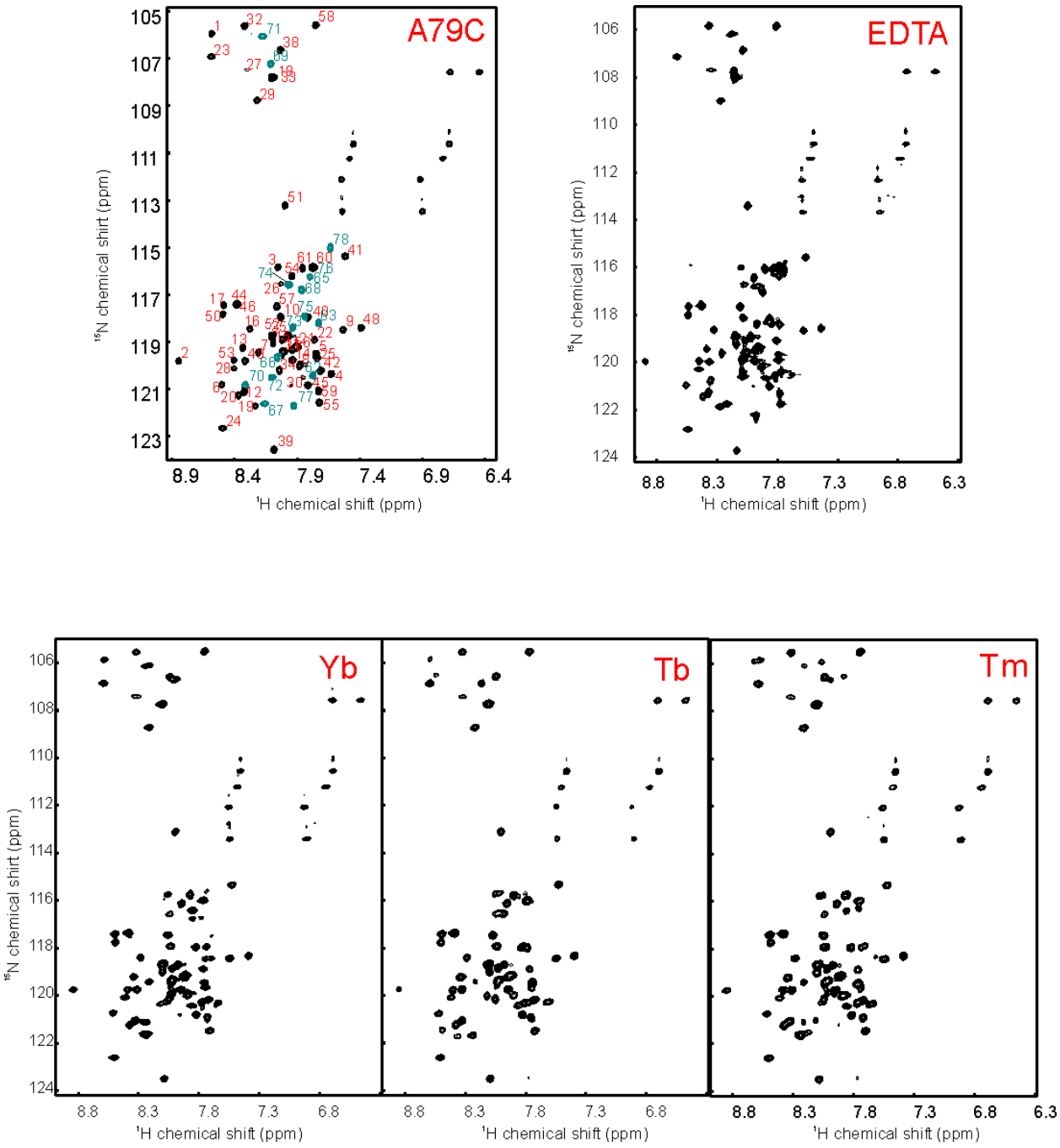

Figure S2. HSQC spectra of A79C mutant subunit c. Clockwise from top left are unmodified A79C, A79C with EDTA modification and no bound metal, and modified with Tm^{3+}, Tb^{3+}, or Yb^{3+}. Assignments are shown in the first panel, with red labels indicating resonances that do not shift on addition of lanthanide, and green identifying those that do.

Table S1. Measured $^{1}H^{15}N$ and $^{13}C'^{13}C^{\alpha}$ RDC values for A79C with bound lanthanide ions.

Residue	Tm^{3+} at 800 MHz		Yb^{3+} at 800 MHz		Tb^{3+} at 900 MHz	
	HN	$C'C^{\alpha}$	HN	$C'C^{\alpha}$	HN	$C'C^{\alpha}$
1		-0.2		-3.9		1.6
2	3.4	1.3	0.3	0.1	2.0	-0.1
3	-2.3	1.7	2.2	0.9	-4.1	1.1
4	-1.1	-3.8	-1.3	0.2	0.4	1.8
5	-0.1	2.6	2.0	0.4	2.4	0.9
6	-1.0	-0.6	2.0	-0.4	0.1	-3.4
7	0.6	-7.3	-0.6	0.0	0.7	1.1
8	-4.6	-1.8	-0.1	0.1	-5.0	0.8
9	-0.7	-0.5	-1.1	1.9	2.9	2.2
10	2.8	8.9	-3.4	4.4	5.9	3.0
11	-8.3	0.8	n.d.	2.1	n.d.	2.8
12	-6.4	0.9	-1.5	-0.1	-1.4	-4.3
13	-0.6	-1.1	-1.6	1.0	-3.1	2.3
14	-5.7	n.d.	2.3	n.d.	-0.8	n.d.
15	6.1	-5.2	-2.4	-0.4	-0.2	1.8
16	2.7	3.9	2.8	-0.8	-0.8	3.6
17	-1.5	3.1	-0.4	0.5	3.3	8.6
18	3.7	-2.9	4.3	-5.7	-2.0	-6.0
19	0.7	1.7	4.7	0.0	0.1	1.2
20	-1.8	3.4	-1.2	3.1	1.9	-4.1
21	-1.2	n.d.	5.1	n.d.	2.8	-0.6
22	-5.2	0.7	2.4	0.3	-6.8	5.8
23	3.9	-1.9	0.5	-0.5	2.7	-1.7
24	-2.3	n.d.	2.6	n.d.	-1.5	n.d.
25	5.7	n.d.	-0.3	n.d.	1.8	n.d.
26	-10.6	n.d.	-3.8	-2.2	-8.1	n.d.
27	5.0	-6.3	n.d.	-1.7	0.0	-8.2
28	-2.0	-0.9	2.3	-0.5	3.5	-4.3
29	-0.1	-1.8	5.2	3.3	0.1	5.2
30	-1.5	-2.0	-1.2	3.7	-2.7	-5.6
31	-1.9	-2.0	2.2	0.2	1.6	-3.9
32	1.3	0.9	2.4	-0.5	1.9	-2.3
33	2.6	1.0	1.8	-0.2	3.5	1.3
34	-2.4	-0.1	0.9	-0.4	-4.0	1.5
35	-0.1	1.9	-5.1	1.0	-1.0	0.1
36	1.3	2.3	0.8	0.4	-1.8	0.7
37	0.9	0.9	-0.4	0.4	0.5	-0.5
38	1.4	-0.7	5.3	-0.3	3.5	-4.5
39	-4.1	-0.4	-2.4	-0.1	-2.2	0.0
40	1.2	-1.3	-0.3	0.3	1.3	-1.8
41	-0.6	1.6	0.5	0.3	-2.8	-1.2
42	1.8	n.d.p.	2.8	n.d.p.	-2.4	n.d.p.
43	n.d.p.	-0.9	n.d.p.	0.1	n.d.p.	1.5
44	-0.8	-0.7	-1.1	0.4	3.0	-0.5
45	0.1	4.0	-2.0	1.7	0.0	3.3
46	-0.8	n.d.p.	3.3	n.d.p.	-3.2	n.d.p.
47	n.d.p.	2.6	n.d.p.	0.3	n.d.p.	-0.9
48	0.7	-1.8	2.4	0.7	-1.3	-4.2
49	-1.8	-2.5	-1.0	-1.8	1.8	-1.8

50	1.7	0.6	2.4	0.5	5.1	0.3
51	0.1	-7.4	3.0	0.3	-0.6	-1.0
52	-2.5	-1.4	0.0	-1.3	2.7	-1.8
53	-2.5	3.5	2.8	2.4	-1.0	-5.2
54	-0.3	-2.2	-2.4	0.8	1.3	1.4
55	-5.3	2.2	-3.3	3.0	-1.6	-0.3
56	3.1	0.6	0.1	-0.3	1.2	-1.8
57	-2.4	0.7	-0.8	-0.4	-2.7	1.1
58	2.8	-0.9	-1.6	-0.2	1.5	-4.0
59	-2.9	-2.4	-6.6	-1.2	-0.8	-1.5
60	-4.1	0.6	-3.5	1.1	-2.1	3.5
61	-2.5	0.6	-2.1	0.4	-1.6	-8.9
62	4.4	-5.6	-1.9	n.d.	1.8	3.6
63	1.9	n.d.p.	n.d.	n.d.p.	-5.7	n.d.p.
64	n.d.p.	n.d.	n.d.p.	n.d.	n.d.p.	n.d.
65	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
66	n.a.	n.a.	1.4	n.a.	n.a.	2.8
67	1.4	n.a.	n.a.	n.a.	0.5	4.8
68	-2.1	n.a.	-1.7	n.a.	-1.2	-1.9
69	n.a.	n.a.	n.a.	n.a.	2.0	n.a.
70	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
71	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
72	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
73	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
74	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
75	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
76	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
77	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
78	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
79	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. – Not assigned due to large pseudocontact shift.
n.d. – Not determined due to low signal to noise ratio.
n.d.p. – Not assigned due to proline.

Methods

Preparation of A79C. The mutation was generated using the Quickchange Site Directed Mutagenesis Kit (Stratagene). Purification and sample preparation was carried out as described[1-3], with some modifications. Protein samples in 1:1 $CHCl_3$:CH_3OH with 15 mg of LPPG were dried under a stream of argon. It was necessary to add DTT to 3-5 mM in order to prevent intermolecular disulfide bond formation.

(1) Girvin, M. E.; Fillingame, R. H. *Biochemistry* **1995**, *34*, 1635-1645.
(2) Girvin, M. E.; Rastogi, V. K.; Abildgaard, F.; Markley, J. L.; Fillingame, R. H. *Biochemistry* **1998**, *37*, 8817-8824.
(3) Krueger-Koplin, R. D.; Sorgen, P. L.; Krueger-Koplin, S. T.; Rivera-Torres, I. O.; Cahill, S. M.; Hicks, D. B.; Grinius, L.; Krulwich, T. A.; Girvin, M. E. *J Biomol NMR* **2004**, *28*, 43-57.