Supporting Information

Domain cooperativity in multidomain proteins: what can we learn from molecular alignment in anisotropic media?

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Construct	Forward primer	Reverse primer	Full amino-acid sequence
dSH3	CATGCCATGGAGCTTGT GCTAGCACTCTATG	ATCGATAAGCTTGGATCCCTAC	MELVLALYDYQEKSPREVTMKKGDILTLLNST NKDWWKVEVNDRQGFVPAAYVKKLD
fSH3	–	–	MDETGKELVLALYDYQEKSPREVTMKKGDILT LLNSTNKDWWKVEVNDRQGFVPAAYVKKLD
dSH3-*sl*-dSH3	N domain: CATGCCATGGAGCTTGT GCTAGCACTCTATG C domain: ACGCTCCGGAGAGCTTG TGCTAGCACTCTATG	N domain: ACGCTCCGGAACCATCTAGTTT TTTCACATAGGCAGC C domain: ATCGATAAGCTTGGATCCCTAC	MELVLALYDYQEKSPREVTMKKGDILTLLNST NKDWWKVEVNDRQGFVPAAYVKKLDGSGEL VLALYDYQEKSPREVTMKKGDILTLLNSTNKD WWKVEVNDRQGFVPAAYVKKLD
dSH3-*ml*-dSH3	N domain: CATGCCATGGAGCTTGT GCTAGCACTCTATG C domain: ACGCTCCGGAGGTGGTG GTGAGCTTGTGCTAGCA CTCTATG	N domain: ACGCTCCGGAACCACCACCAC CATCTAGTTTTTTCACATAGGC AGC C domain: ATCGATAAGCTTGGATCCCTAC	MELVLALYDYQEKSPREVTMKKGDILTLLNST NKDWWKVEVNDRQGFVPAAYVKKLDGGGG SGGGGELVLALYDYQEKSPREVTMKKGDILTL LNSTNKDWWKVEVNDRQGFVPAAYVKKLD
dSH3-*ll*-dSH3	N domain: CATGCCATGGAGCTTGT GCTAGCACTCTATG C domain: ACGCTCCGGAGGCGGA GGCTCAGGTGGTGGTG GTTCTGGAGGTGGTGGT GAGCTTG	N domain: ACGCTCCGGACCCGCCTCCGC CTGAACCACCACCACCGGAAC CACCACCACCATCTAG C domain: ATCGATAAGCTTGGATCCCTAC	MELVLALYDYQEKSPREVTMKKGDILTLLNST NKDWWKVEVNDRQGFVPAAYVKKLDGGGG SGGGGSGGGGSGGGGSGGGGSGGGGELVL ALYDYQEKSPREVTMKKGDILTLLNSTNKDW WKVEVNDRQGFVPAAYVKKLD

Tab. S1. DNA primer sequences used in production of α-spectrin SH3 constructs and amino-acid sequences of the constructs. DNA restriction sites are marked in red, disordered residues marked in blue. fSH3 plasmid [Musacchio *et al*. *Nature* **1992,** *359*, 851] was used as a PCR template to construct dSH3. In turn, dSH3 plasmid was used as a template for dSH3-*sl*-dSH3 and dSH3-*ml*-dSH3 and, finally, dSH3-*ml*-dSH3 was used as a template for dSH3-*ll*-dSH3.

Residue	dSH3	dSH3	fSH3	fSH3 (high salt)	dSH3-*sl*-dSH3	dSH3-*sl*-dSH3	dSH3-*ml*-dSH3	dSH3-*ll*-dSH3	dSH3-*ll*-dSH3
9	w	-32.4	-9.4	-20.0	12.5	12.0	2.8	-0.2	-0.5
10	w	w	-16.0	w	15.4	14.6	7.8	-0.5	-1.3
11	4.6	5.1	2.5	5.5	15.8	12.5	7.0	8.5	9.7
12	0.5	2.5	0.2	w	w	w	w	-2.8	-4.1
13	w	s	-2.3	0.0	9.9	9.3	6.7	5.7	6.5
14	-11.9	-18.0	-0.5	5.2	15.0	14.8	9.5	7.7	8.9
15	w	w	11.7	17.8	15.1	14.9	9.8	11.9	13.9
16	14.6	11.8	10.4	16.8	22.9	22.1	10.8	13.3	15.4
17	w	34.2	12.5	21.9	1.1	1.3	1.5	8.3	8.9
18	w	w	-25.0	w	4.3	4.0	-1.1	-10.0	-11.8
19	w	w	19.7	32.4	-23.0	-22.4	-9.9	-4.0	-5.1
20	p	p	p	p	p	p	p	p	p
21	32.4	w	15.3	24.8	4.5	4.8	4.9	7.3	8.9
22	-4.6	-2.0	-10.7	-20.4	-13.0	-13.2	-6.8	-11.6	-13.0
23	w	w	-7.2	-7.0	16.3	16.1	6.3	3.0	2.6
24	-6.6	-16.2	1.9	2.8	19.3	18.6	10.1	9.8	11.1
25	25.1	33.4	14.1	w	14.1	13.7	8.4	11.1	12.7
26	25.1	24.3	8.8	w	13.3	12.4	9.1	5.8	6.5
27	5.7	6.7	-5.6	-10.3	4.0	3.1	1.9	-2.1	-3.4
28	w	w	-14.7	w	2.3	3.4	-0.7	-3.0	-3.0
29	36.7	o	17.0	25.9	-15.9	-15.4	o	1.3	2.0
30	-19.9	-22.8	-9.2	w	o	o	o	3.0	3.2
31	-16.3	-23.5	-5.6	-11.9	16.7	16.1	7.4	7.1	4.8
32	-26.3	-22.2	-9.4	-21.2	-2.7	-3.5	-3.9	-5.4	-6.2
33	w	w	13.5	w	-26.1	-26.5	w	-2.0	-2.0
34	19.7	17.9	-6.1	w	4.3	7.0	3.0	-0.6	-1.5
35	w	w	-21.8	w	10.1	9.8	0.6	-2.3	-2.9
36	-15.0	-20.0	-14.1	-23.4	10.1	9.6	2.7	-2.1	-3.6
37	-11.7	-20.0	0.2	-2.6	18.4	17.5	9.3	6.9	8.4
38	-14.2	-14.5	-7.9	-13.7	9.5	9.2	4.0	2.7	2.9
39	8.1	o	3.2	o	-17.2	-15.6	o	-2.8	-2.7
40	5.4	4.8	o	o	-12.4	-12.1	-5.3	-0.4	-0.1
41	-35.2	w	-20.2	w	-0.2	0.1	-2.8	-6.7	-7.7
42	w	w	-23.7	w	7.4	7.5	3.0	-4.1	-4.8
43	w	w	o	w	5.2	5.4	-1.3	-10.3	-13.1
44	8.0	w	-1.2	-4.5	-13.6	-13.0	-9.8	-9.9	-11.6
45	o	o	6.6	o	o	o	o	o	o
46	s	s	o	w	-26.9	-26.1	-13.3	-8.5	-9.6
47	28.7	31.8	12.2	21.2	-21.6	-21.5	-11.3	-0.7	-1.9
48	-12.1	-10.8	-9.6	-17.5	-5.7	-5.8	-5.9	-7.7	-8.7
49	9.2	8.1	12.9	24.9	o	o	o	-0.6	-2.1
50	8.4	8.3	10.0	16.6	o	o	o	o	o
51	17.8	26.4	9.0	13.2	-23.9	-23.1	-11.9	-6.3	-6.5
52	26.4	30.0	10.2	17.6	-22.7	-22.6	-9.7	-6.8	-8.7
53	-34.5	-36.4	-27.4	w	4.8	4.7	-0.4	-9.4	-11.9
54	p	p	p	p	p	p	p	p	p
55	w	w	15.5	w	-27.4	-25.7	-11.1	-4.9	-6.3
56	40.9	w	23.4	41.5	-29.7	-29.7	-11.6	-4.7	-6.4
57	w	w	12.7	w	-8.0	-8.5	-3.1	2.6	3.3
58	s	s	-8.8	-13.9	-2.9	-2.1	-3.7	-2.1	-2.4
59	o	o	-11.8	o	o	o	o	o	o
60	0.1	-0.2	-0.3	-1.3	12.1	11.9	5.3	6.3	6.8

Tab. S2. Experimentally measured RDCs in various constructs of α-spc SH3 domain. For tandems, each value represents the average between the N- and C-terminal sites. Sample conditions are pH 3.5, 20 mM sodium citrate, 5% PEG/hexanol (r=0.85); high salt sample additionally contains 100 mM NaCl. Abbreviations: (w) weak peak, (o) overlapped peak, (p) proline residue, (s) misshaped peak.

Residue	dSH3	fSH3	dSH3-*sl*-dSH3	dSH3-*ml*-dSH3	dSH3-*ll*-dSH3
9	-22.3	-5.2	12.6	4.2	-0.6
10	-27.9	-8.4	14.4	11.1	-0.8
11	-0.5	1.4	12.5	7.7	5.5
12	2.2	0.1	w	w	-2.1
13	-16.4	-0.8	9.4	6.0	3.7
14	-12.1	0.3	14.4	9.7	5.0
15	15.9	7.0	14.3	10.7	7.5
16	8.9	6.2	22.1	12.9	8.4
17	24.5	7.0	1.4	2.3	5.8
18	w	-14.7	4.8	-1.0	-7.4
19	43.4	10.1	-22.7	-12.0	-1.7
20	p	p	p	p	p
21	30.0	8.4	4.1	5.7	5.1
22	-1.7	-6.3	-12.7	-6.6	-7.7
23	-19.0	-4.5	15.8	7.2	1.4
24	-8.5	0.4	17.9	10.5	5.7
25	25.9	7.7	13.2	10.4	6.9
26	17.9	5.4	11.0	8.5	4.0
27	4.9	-2.9	3.3	1.5	-2.1
28	-19.5	-8.5	3.9	-0.1	-1.9
29	39.3	9.0	-15.1	o	0.7
30	-17.7	-5.1	o	o	1.6
31	-19.9	-3.2	14.4	8.3	4.5
32	-16.0	-5.5	-3.3	-4.2	-4.8
33	35.7	6.5	-26.5	-10.2	-1.1
34	2.9	-3.9	w	2.6	-0.2
35	-30.2	-11.8	9.3	3.4	-1.5
36	-11.5	-7.8	8.6	2.3	-2.3
37	-21.4	0.5	17.8	8.8	4.6
38	-11.4	-3.9	9.3	3.5	1.5
39	9.0	1.5	-16.6	o	-0.7
40	4.1	o	-11.0	-4.0	0.5
41	-28.0	-11.0	-0.4	-3.1	-4.5
42	w	-13.0	7.5	1.7	-3.1
43	w	-16.6	5.2	-1.6	-7.5
44	16.9	-1.1	-13.4	-7.9	-6.9
45	o	2.9	o	o	o
46	20.1	8.4	-27.3	-15.8	-5.1
47	22.9	6.1	-19.7	-11.1	-2.7
48	-8.3	-6.2	-6.4	-6.1	-4.4
49	6.5	6.9	o	o	-0.1
50	6.0	5.2	o	o	o
51	20.6	4.2	-23.1	-12.4	-3.7
52	21.5	5.2	-20.1	-9.3	-4.3
53	-26.6	-14.8	4.3	-1.3	-6.7
54	p	p	p	p	p
55	30.1	7.8	-26.9	-19.1	-1.4
56	46.4	12.6	-29.3	-15.2	-2.0
57	7.6	6.8	-8.3	-2.5	2.4
58	-8.8	-5.0	-2.3	-3.6	-1.6
59	o	-6.3	o	o	o
60	-0.2	0.2	11.8	6.5	3.6

Tab. S2. Experimentally measured RDCs in various constructs of α-spc SH3 domain. For tandems, each value represents the average between the N- and C-terminal sites. Sample conditions are pH 3.5, 20 mM sodium citrate, 5% PEG/hexanol (r=0.96).

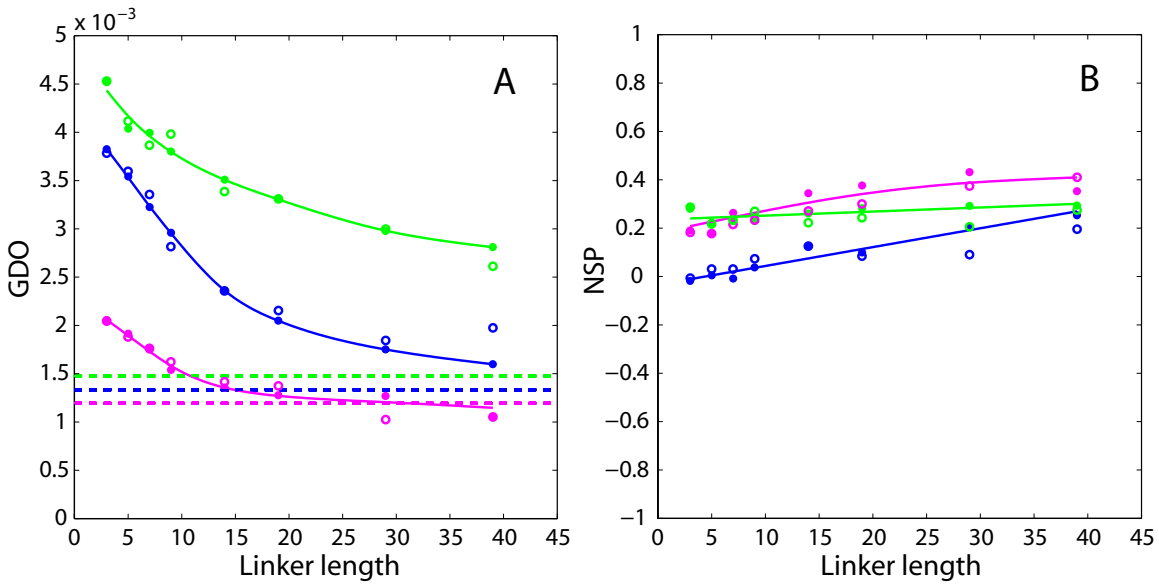

Fig. S1. PALES simulations of steric alignment in a series of computer-generated tandem proteins. The plot reproduces the results from Fig. 3 (closed circles) and, in addition, contains the results from the equivalent series of simulations using independently generated structural ensembles (open circles).