wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	2MAB
Title	:	Untangling the Solution Structure of C-Terminal Domain of Aciniform Spidroin
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This is a wwPDB NMR Structure Validation Summary Report for a publicly released PDB entry.
We welcome your comments at validation@mail.wwpdb.org
A user guide is available at
http://wwpdb.org/validation/2016/NMRValidationReportHelp
with specific help available everywhere you see the (i) symbol.

The following versions of software and data (see references (i)) were used in the production of this report:

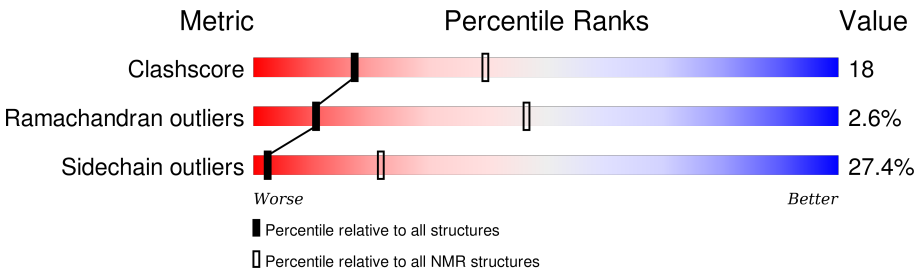
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	unknown
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	rb-20027457
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	rb-20027457

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
SOLUTION NMR

The overall completeness of chemical shifts assignment is 42%.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	NMR archive (#Entries)
Clashscore	114402	11133
Ramachandran outliers	111179	9975
Sidechain outliers	111093	9958

The table below summarises the geometric issues observed across the polymeric chains and their fit to the experimental data. The red, orange, yellow and green segments indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria. A cyan segment indicates the fraction of residues that are not part of the well-defined cores, and a grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5%

Mol	Chain	Length	Quality of chain
1	A	109	52% 39% 6% •
1	B	109	45% 48% 5% •

2 Ensemble composition and analysis (i)

This entry contains 20 models. Model 14 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *fewest violations*.

The following residues are included in the computation of the global validation metrics.

Well-defined (core) protein residues			
Well-defined core	**Residue range (total)**	**Backbone RMSD (Å)**	**Medoid model**
1	A:4-A:108, B:4-B:109 (211)	0.14	14

Ill-defined regions of proteins are excluded from the global statistics.

Ligands and non-protein polymers are included in the analysis.

The models can be grouped into 4 clusters and 1 single-model cluster was found.

Cluster number	Models
1	1, 2, 3, 4, 7, 10, 14, 15, 16, 20
2	5, 6, 9, 13, 19
3	8, 17
4	11, 18
Single-model clusters	12

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 3100 atoms, of which 1576 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called ACINIFORM SPIDROIN.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	109	1551	468	789	136	157	1	0
1	B	109	1549	468	787	136	157	1	0

4 Residue-property plots (i)

4.1 Average score per residue in the NMR ensemble

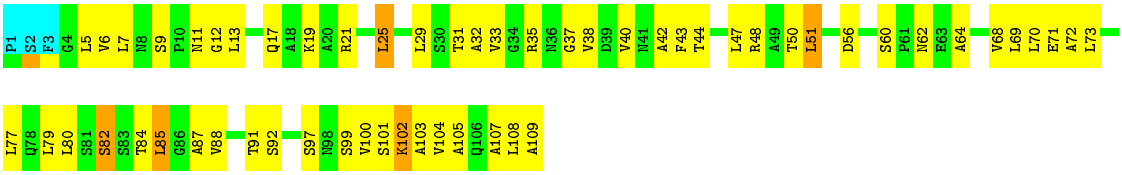
These plots are provided for all protein, RNA and DNA chains in the entry. The first graphic is the same as shown in the summary in section 1 of this report. The second graphic shows the sequence where residues are colour-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. Stretches of 2 or more consecutive residues without any outliers are shown as green connectors. Residues which are classified as ill-defined in the NMR ensemble, are shown in cyan with an underline colour-coded according to the previous scheme. Residues which were present in the experimental sample, but not modelled in the final structure are shown in grey.

- Molecule 1: ACINIFORM SPIDROIN

Chain A: 52% 39% 6% •

- Molecule 1: ACINIFORM SPIDROIN

Chain B: 45% 48% 5% •



4.2 Residue scores for the representative (medoid) model from the NMR ensemble

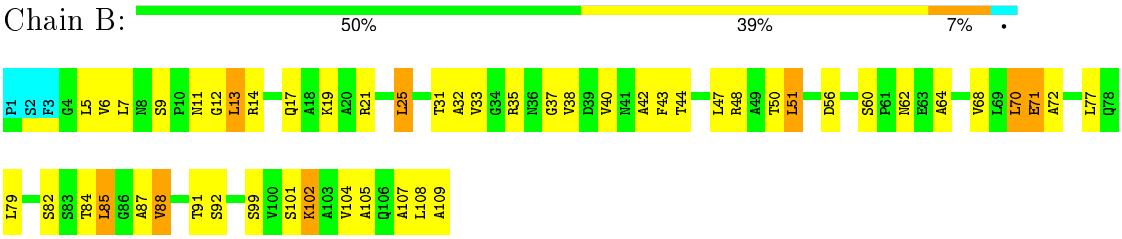
The representative model is number 14. Colouring as in section 4.1 above.

- Molecule 1: ACINIFORM SPIDROIN

Chain A: 53% 36% 7% •

- Molecule 1: ACINIFORM SPIDROIN

Chain B:


50%
39%
7%
P1 S2 F3 G4 L5 V6 L7 N8 S9 P10 N11 G12 L13 R14 Q17 A18 K19 A20 R21 L25 T31 A32 V33 G34 R35 N36 G37 V38 D39 V40 N41 A42 F43 T44 L47 R48 A49 T50 L51 D56 S60 P61 N62 E63 A64 V68 L69 L70 E71 A72 L77 Q78
L79 S82 S83 T84 L85 G86 A87 V88 T91 S92 S99 V100 S101 K102 A103 V104 A105 Q106 A107 L108 A109

5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *simulated annealing*.

Of the 20 calculated structures, 20 were deposited, based on the following criterion: *target function*.

The following table shows the software used for structure solution, optimisation and refinement.

Software name	Classification	Version
CYANA	structure solution	
CYANA	refinement	

The following table shows chemical shift validation statistics as aggregates over all chemical shift files. Detailed validation can be found in section 7 of this report.

Chemical shift file(s)	2mab_cs.str
Number of chemical shift lists	1
Total number of shifts	1188
Number of shifts mapped to atoms	1188
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	42%

No validations of the models with respect to experimental NMR restraints is performed at this time.

6 Model quality (i)

6.1 Standard geometry (i)

There are no covalent bond-length or bond-angle outliers.

There are no bond-length outliers.

There are no bond-angle outliers.

There are no chirality outliers.

There are no planarity outliers.

6.2 Too-close contacts (i)

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in each chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes averaged over the ensemble.

Mol	Chain	Non-H	H(model)	H(added)	Clashes
1	A	733	761	761	35±5
1	B	738	766	766	36±3
All	All	29420	30540	30540	1074

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including hydrogen atoms). The all-atom clashscore for this structure is 18.

5 of 163 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:B:32:ALA:HB2	1:B:42:ALA:HB3	0.85	1.46	8	20
1:A:32:ALA:HB2	1:A:42:ALA:HB3	0.84	1.49	11	18
1:B:33:VAL:HG22	1:B:38:VAL:HG23	0.74	1.60	5	1
1:A:104:VAL:HG21	1:B:70:LEU:HD21	0.69	1.62	15	8
1:A:87:ALA:HB3	1:B:82:SER:HB2	0.68	1.66	18	16

6.3 Torsion angles (i)

6.3.1 Protein backbone (i)

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all NMR

entries. The Analysed column shows the number of residues for which the backbone conformation was analysed and the total number of residues.

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles
1	A	105/109 (96%)	94±1 (90±1%)	8±1 (7±1%)	3±0 (3±0%)	10 45
1	B	105/109 (96%)	95±1 (91±1%)	7±1 (7±1%)	3±0 (2±0%)	11 48
All	All	4200/4360 (96%)	3792 (90%)	300 (7%)	108 (3%)	11 47

5 of 6 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	A	38	VAL	20
1	A	12	GLY	20
1	B	12	GLY	20
1	B	38	VAL	19
1	A	88	VAL	16

6.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all NMR entries. The Analysed column shows the number of residues for which the sidechain conformation was analysed and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	A	81/84 (96%)	60±1 (73±2%)	22±1 (27±2%)	3 23
1	B	81/84 (96%)	58±2 (72±2%)	23±2 (28±2%)	2 20
All	All	3240/3360 (96%)	2352 (73%)	888 (27%)	2 22

5 of 70 unique residues with a non-rotameric sidechain are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	B	102	LYS	20
1	A	35	ARG	20
1	B	9	SER	20
1	A	51	LEU	20
1	A	85	LEU	20

6.3.3 RNA (i)

There are no RNA molecules in this entry.

6.4 Non-standard residues in protein, DNA, RNA chains (i)

There are no non-standard protein/DNA/RNA residues in this entry.

6.5 Carbohydrates (i)

There are no carbohydrates in this entry.

6.6 Ligand geometry (i)

There are no ligands in this entry.

6.7 Other polymers (i)

There are no such molecules in this entry.

6.8 Polymer linkage issues (i)

There are no chain breaks in this entry.

7 Chemical shift validation (i)

The completeness of assignment taking into account all chemical shift lists is 42% for the well-defined parts and 42% for the entire structure.

7.1 Chemical shift list 1

File name: 2mab_cs.str

Chemical shift list name: *assigned_chem_shift_list_1*

7.1.1 Bookkeeping (i)

The following table shows the results of parsing the chemical shift list and reports the number of nuclei with statistically unusual chemical shifts.

Total number of shifts	1188
Number of shifts mapped to atoms	1188
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	3

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	109	-0.77 ± 0.12	Should be applied
$^{13}C_{\beta}$	100	-0.07 ± 0.04	None needed (< 0.5 ppm)
$^{13}C'$	0	—	—
^{15}N	105	0.05 ± 0.20	None needed (< 0.5 ppm)

7.1.3 Completeness of resonance assignments (i)

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 42%, i.e. 961 atoms were assigned a chemical shift out of a possible 2277. 25 out of 52 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	413/1043 (40%)	206/416 (50%)	105/422 (25%)	102/205 (50%)
Sidechain	541/1216 (44%)	324/697 (46%)	201/465 (43%)	16/54 (30%)

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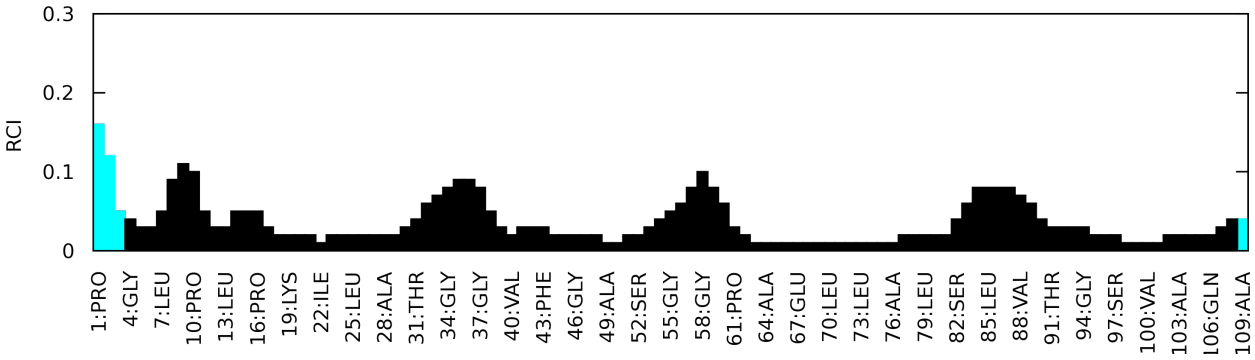
	Total	^{1}H	^{13}C	^{15}N
Aromatic	7/18 (39%)	5/10 (50%)	2/8 (25%)	0/0 (—%)
Overall	961/2277 (42%)	535/1123 (48%)	308/895 (34%)	118/259 (46%)

7.1.4 Statistically unusual chemical shifts (i)

The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	67	GLU	CG	26.64	42.24 – 29.94	-7.7
1	A	71	GLU	CG	27.96	42.24 – 29.94	-6.6
1	A	78	GLN	CG	27.50	39.38 – 28.18	-5.6

7.1.5 Random Coil Index (RCI) plots (i)

The image below reports *random coil index* values for the protein chains in the structure. The height of each bar gives a probability of a given residue to be disordered, as predicted from the available chemical shifts and the amino acid sequence. A value above 0.2 is an indication of significant predicted disorder. The colour of the bar shows whether the residue is in the well-defined core (black) or in the ill-defined residue ranges (cyan), as described in section 2 on ensemble composition.

Random coil index (RCI) for chain A: