wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	1MKE
Title	:	Structure of the N-WASP EVH1 Domain-WIP complex
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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:

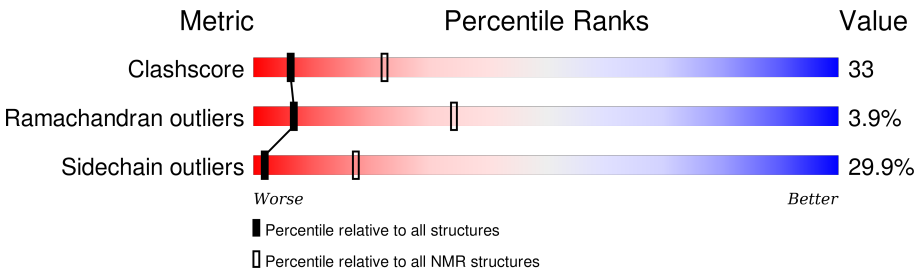
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	unknown
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	rb-20027457
Ideal 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 87%.

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	152	23% 44% 6% 22% 5%

2 Ensemble composition and analysis (i)

This entry contains 21 models. Model 15 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *minimized average structure*.

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:2-A:8, A:14-A:18, A:40-A:72, A:77-A:142 (111)	0.42	15

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 5 clusters and 2 single-model clusters were found.

Cluster number	Models
1	1, 3, 7, 8, 9, 13, 15
2	4, 14, 18, 21
3	5, 12, 19
4	2, 6, 16
5	10, 20
Single-model clusters	11; 17

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 2281 atoms, of which 1132 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Fusion protein consisting of Wiskott-Aldrich syndrome protein interacting protein (WIP), GSGSG linker, and Neural Wiskott-Aldrich syndrome protein (N-WASP).

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	144	2281	729	1132	201	212	7	0

There are 5 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	26	GLY	-	LINKER	UNP O43516
A	27	SER	-	LINKER	UNP O43516
A	28	GLY	-	LINKER	UNP O43516
A	29	SER	-	LINKER	UNP O43516
A	30	GLY	-	LINKER	UNP O43516

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: Fusion protein consisting of Wiskott-Aldrich syndrome protein interacting protein (WIP), GSGSG linker, and Neural Wiskott-Aldrich syndrome protein (N-WASP)

Chain A: 23% 44% 6% 22% 5%

D1 L2 P3 E6 F7 Y8 V9 Q10 T11 T12 K13 S14 Y15 L19 A20 R21 N22 E23 S24 R25 G26 S27 G28 S29 G30 S31 L32 F33 S34 F35 L36 G37 K38 K39 M43 A46 V47 V48 Q49 L50 Y51 A52 A53 D54 R55 N56 C57 M58 W59 S60 K61 K62 C63 S64 G65 V66

A67 C68 L69 V70 K71 D72 N73 P74 Q75 R76 S77 Y78 F79 L80 R81 I82 F83 D84 I85 K86 L90 L91 W92 E93 Q94 E95 L96 Y97 N98 N99 F100 V101 Y102 R106 G107 Y108 F109 H110 T111 F112 A113 G114 D115 T116 C117 Q118 V119 A120 L121 N122 F123 E128 A129 K130 K131 F132 R133

K134 T137 D138 L139 L140 G141 R142 R143 Q144 ARG LYS SER GLU LYS ARG ARG ASP

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

The representative model is number 15. Colouring as in section 4.1 above.

• Molecule 1: Fusion protein consisting of Wiskott-Aldrich syndrome protein interacting protein (WIP), GSGSG linker, and Neural Wiskott-Aldrich syndrome protein (N-WASP)

Chain A: 28% 38% 7% 22% 5%

D1 L2 P3 P4 P5 E6 P7 Y8 V9 Q10 T11 T12 K13 L19 A20 R21 N22 E23 S24 R25 G26 S27 G28 S29 G30 S31 L32 F33 S34 F35 L36 G37 K38 K39 T42 M43 A46 V47 V48 Q49 L50 Y51 A52 A53 D54 R55 N56 C57 M58 W59 S60 K61 K62 C63 S64 G65 V66

A67 C68 L69 V70 K71 D72 N73 P74 Q75 R76 S77 Y78 F79 L80 R81 I82 F83 D84 I85 K86 K89 L90 L91 W92 E93 Q94 E95 L96 F100 V101 Y102 N103 R106 G107 Y108 F109 H110 T111 F112 A113 C117 A120 L121 N122 F123 A124 N125 E128 A129 K130 K131 F132 R133 K134

A135 V136 T137 D138 L139 L140 G141 R142 R143 Q144 ARG LYS SER GLU LYS ARG ARG ASP

5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *torsion angle dynamics*.

Of the 50 calculated structures, 21 were deposited, based on the following criterion: *LOWEST TARGET FUNCTION*.

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

Software name	Classification	Version
DYANA	structure solution	1.5
DYANA	refinement	1.5

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)	BMRB entry 5554
Number of chemical shift lists	1
Total number of shifts	1492
Number of shifts mapped to atoms	1492
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	87%

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	893	872	872	58±6
All	All	18753	18312	18312	1220

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 33.

5 of 444 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:47:VAL:HG13	1:A:124:ALA:HB3	1.01	1.32	6	3
1:A:82:ILE:HD12	1:A:92:TRP:CE3	0.97	1.93	14	21
1:A:84:ASP:N	1:A:91:LEU:HD21	0.93	1.78	14	2
1:A:100:PHE:O	1:A:140:LEU:HD21	0.92	1.64	13	17
1:A:69:LEU:HD12	1:A:80:LEU:HD21	0.90	1.40	17	1

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	111/152 (73%)	97±2 (87±2%)	10±2 (9±2%)	4±2 (4±2%)	7 34
All	All	2331/3192 (73%)	2030 (87%)	210 (9%)	91 (4%)	7 34

5 of 16 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	57	CYS	13
1	A	63	CYS	13
1	A	96	LEU	11
1	A	115	ASP	10
1	A	54	ASP	9

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	97/133 (73%)	68±3 (70±3%)	29±3 (30±3%)	2 17
All	All	2037/2793 (73%)	1427 (70%)	610 (30%)	2 17

5 of 72 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	A	102	TYR	21
1	A	83	PHE	21
1	A	121	LEU	20
1	A	106	ARG	20
1	A	61	LYS	19

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 87% for the well-defined parts and 74% for the entire structure.

7.1 Chemical shift list 1

File name: BMRB entry 5554

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	1492
Number of shifts mapped to atoms	1492
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	5

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	126	-0.41 ± 0.10	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	116	-0.20 ± 0.11	None needed (< 0.5 ppm)
$^{13}C'$	108	-0.03 ± 0.20	None needed (< 0.5 ppm)
^{15}N	112	-0.32 ± 0.43	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 87%, i.e. 1206 atoms were assigned a chemical shift out of a possible 1388. 14 out of 18 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	521/543 (96%)	211/216 (98%)	210/222 (95%)	100/105 (95%)
Sidechain	567/695 (82%)	351/412 (85%)	211/250 (84%)	5/33 (15%)

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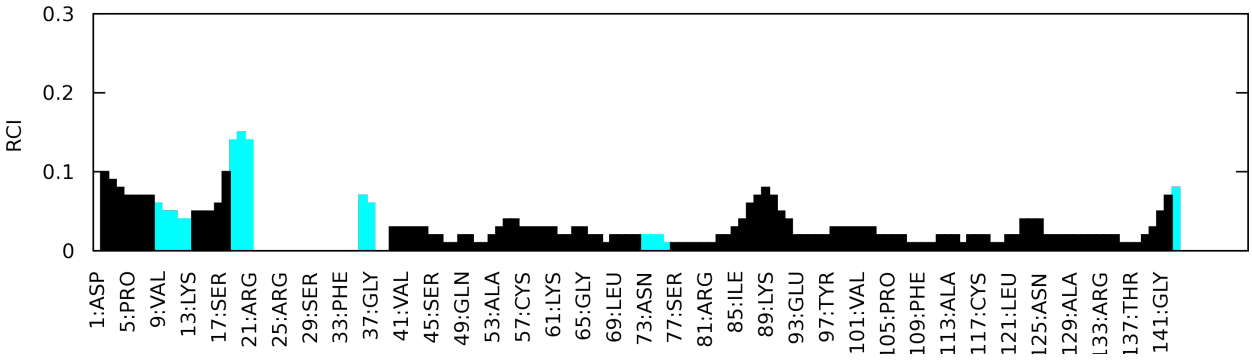
	Total	^{1}H	^{13}C	^{15}N
Aromatic	118/150 (79%)	75/79 (95%)	40/68 (59%)	3/3 (100%)
Overall	1206/1388 (87%)	637/707 (90%)	461/540 (85%)	108/141 (77%)

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	81	ARG	NE	119.90	92.63 – 76.73	22.2
1	A	120	ALA	HB1	-0.44	2.61 – 0.11	-7.2
1	A	120	ALA	HB3	-0.44	2.61 – 0.11	-7.2
1	A	120	ALA	HB2	-0.44	2.61 – 0.11	-7.2
1	A	5	PRO	HG2	-0.15	3.48 – 0.38	-6.7

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: