wwPDB NMR Structure Validation Summary Report (i)

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Title	:	Shotgun proteolysis: A practical application
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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
https://www.wwpdb.org/validation/2017/NMRValidationReportHelp
with specific help available everywhere you see the (i) symbol.

The types of validation reports are described at
http://www.wwpdb.org/validation/2017/FAQs#types.

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

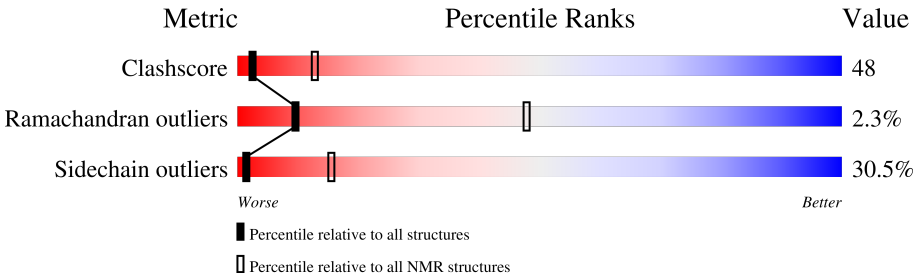
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36.2

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 93%.

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	158937	12864
Ramachandran outliers	154571	11451
Sidechain outliers	154315	11428

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	120	29% 44% 8% 18% •

2 Ensemble composition and analysis (i)

This entry contains 20 models. Model 13 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative.

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:37-A:59, A:78-A:152 (98)	0.35	13

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 2 clusters. No single-model clusters were found.

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

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 1848 atoms, of which 910 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called PUTATIVE MEMBRANE PROTEIN IGAA HOMOLOG.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	119	1848	591	910	156	189	2	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	35	MET	-	expression tag	UNP P45800

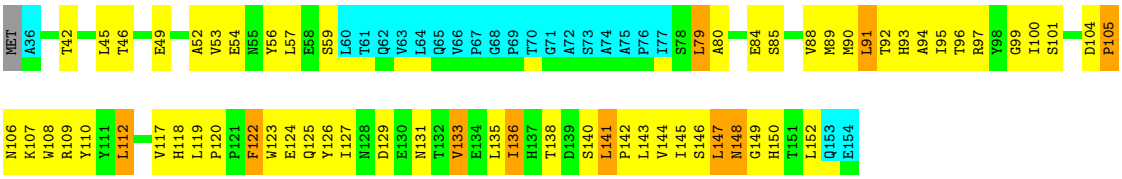
4 Residue-property plots (i)

4.1 Average score per residue in the NMR ensemble

These plots are provided for all protein, RNA, DNA and oligosaccharide 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: PUTATIVE MEMBRANE PROTEIN IGAA HOMOLOG

Chain A: 29% 44% 8% 18%

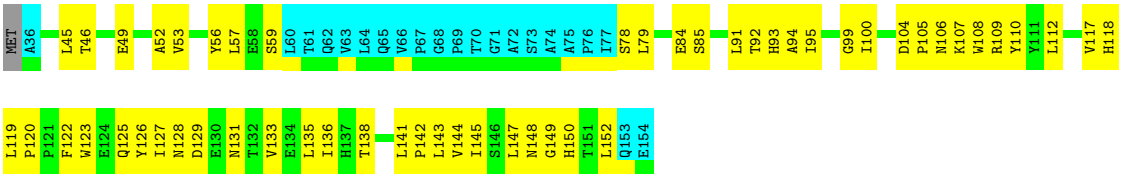


4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 13. Colouring as in section 4.1 above.

• Molecule 1: PUTATIVE MEMBRANE PROTEIN IGAA HOMOLOG

Chain A: 38% 44% 18%



5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *CNS*.

Of the 20 calculated structures, 20 were deposited, based on the following criterion: *NO VIOLATIONS*.

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

Software name	Classification	Version
CNS	refinement	
ANSIG	structure solution	
CNS	structure solution	

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)	working_cs.cif
Number of chemical shift lists	1
Total number of shifts	1502
Number of shifts mapped to atoms	1502
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	93%

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	794	761	757	75±6
All	All	15880	15220	15140	1495

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 48.

5 of 282 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:95:ILE:HG21	1:A:119:LEU:HD13	1.03	1.24	14	8
1:A:88:VAL:HG13	1:A:145:ILE:HD11	0.97	1.33	1	1
1:A:79:LEU:HD23	1:A:141:LEU:HD12	0.96	1.37	7	3
1:A:57:LEU:HD22	1:A:79:LEU:HD21	0.95	1.37	19	1
1:A:57:LEU:HD13	1:A:79:LEU:HD23	0.93	1.39	11	4

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	0	-	-	-	-
All	All	1078/2400 (45%)	973 (90%)	80 (7%)	25 (2%)	9 48

All 3 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	105	PRO	11
1	A	148	ASN	11
1	A	100	ILE	3

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	0	-	-	-
All	All	1260/2120 (59%)	876 (70%)	384 (30%)	1 15

5 of 52 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	42	THR	14
1	A	79	LEU	14
1	A	89	MET	14
1	A	91	LEU	14
1	A	96	THR	14

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 monosaccharides 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 93% for the well-defined parts and 93% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *yrf.dep_3.data.csh*

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	1502
Number of shifts mapped to atoms	1502
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	4

The following errors were found when reading this chemical shift list.

- Chemical shift has been reported more than once. First 5 (of 0) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	57	LEU	HD11	0.783	0.01	2
1	A	57	LEU	HD12	0.783	0.01	2
1	A	57	LEU	HD13	0.783	0.01	2
1	A	81	LEU	HD11	0.731	0.01	2
1	A	81	LEU	HD12	0.731	0.01	2
1	A	81	LEU	HD13	0.731	0.01	2
1	A	129	ASP	HB2	2.641	0.01	1
1	A	130	GLU	HB2	1.763	0.01	2

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	118	-0.20 ± 0.19	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	114	0.10 ± 0.16	None needed (< 0.5 ppm)
$^{13}C'$	118	-0.13 ± 0.13	None needed (< 0.5 ppm)
^{15}N	111	0.08 ± 0.33	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 93%, i.e. 1245 atoms were assigned a chemical shift out of a possible 1345. 0 out of 18 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	480/482 (100%)	193/193 (100%)	194/196 (99%)	93/93 (100%)
Sidechain	670/742 (90%)	468/483 (97%)	202/235 (86%)	0/24 (0%)
Aromatic	95/121 (79%)	51/58 (88%)	44/53 (83%)	0/10 (0%)
Overall	1245/1345 (93%)	712/734 (97%)	440/484 (91%)	93/127 (73%)

7.1.4 Statistically unusual chemical shifts (i)

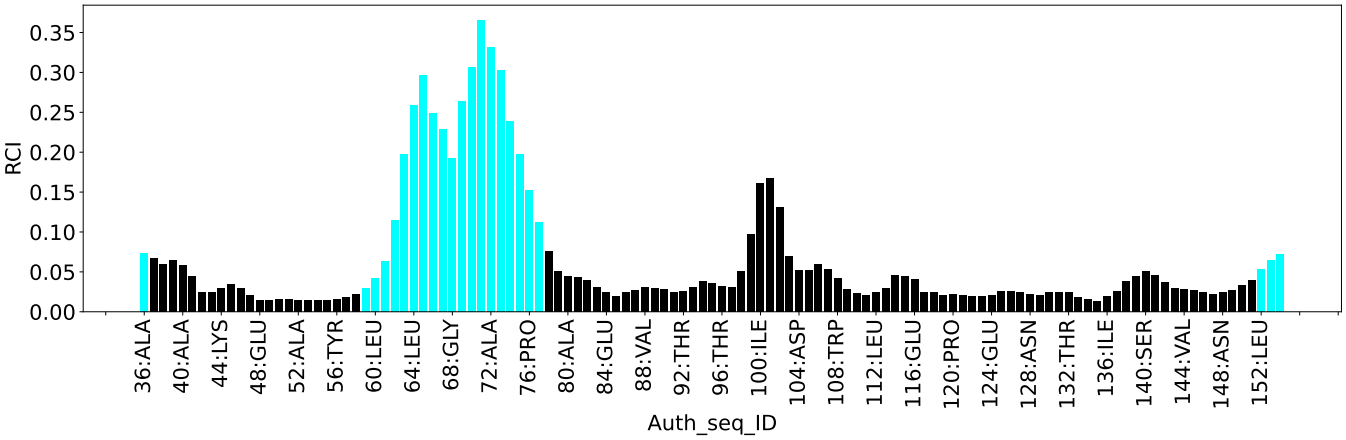
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	138	THR	HG1	5.73	0.08 – 2.19	21.8
1	A	96	THR	HG1	5.42	0.08 – 2.19	20.3
1	A	120	PRO	HB3	-0.37	0.25 – 3.76	-6.8
1	A	125	GLN	HG3	0.82	0.91 – 3.68	-5.3

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.If well-defined core and ill-defined regions are not identified then it is shown as gray bars.

Random coil index (RCI) for chain A:


RCI
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
36:ALA
40:ALA
44:LYS
48:GLU
52:ALA
56:TYR
60:LEU
64:LEU
68:GLY
72:ALA
76:PRO
80:ALA
84:GLU
88:VAL
92:THR
96:THR
100:ILE
104:ASP
108:TRP
112:LEU
116:GLU
120:PRO
124:GLU
128:ASN
132:THR
136:ILE
140:SER
144:VAL
148:ASN
152:LEU
Auth_seq_ID