wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	2KJH
Title	:	NMR based structural model of the UBCH8-UBIQUITIN 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:

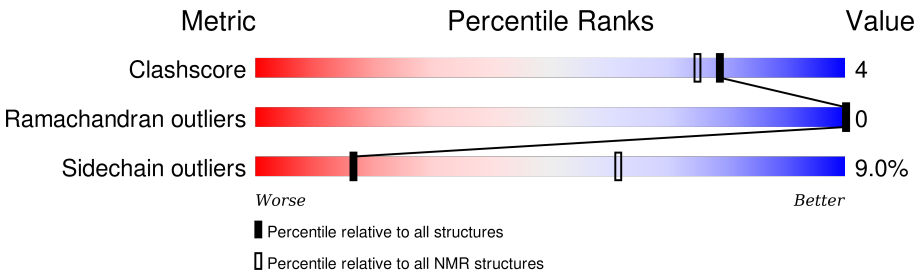
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	unknown
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	rb-20027457
Ideal 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 36%.

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	86% 13% ••
2	B	76	79% 16% 5%

2 Ensemble composition and analysis (i)

This entry contains 16 models. Model 13 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *closest to the mean 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:150 (149)	0.20	13
2	B:1-B:72 (72)	0.15	11

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 3 single-model clusters were found.

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

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 2192 atoms, of which 361 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Ubiquitin/ISG15-conjugating enzyme E2 L6.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	150	1459	792	232	211	218	6	0

- Molecule 2 is a protein called Ubiquitin.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
2	B	76	733	379	129	105	118	2	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	76	CYS	GLY	ENGINEERED	UNP P62988

4 Residue-property plots (i)

4.1 Average score per residue in the NMR ensemble

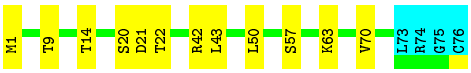
These plots are provided for all protein, RNA and DNA chains in the entry. The first graphic is the same as shown in the summary in section 1 of this report. The second graphic shows the sequence where residues are colour-coded according to the number of geometric quality criteria for which they contain 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: Ubiquitin/ISG15-conjugating enzyme E2 L6

Chain A:

- Molecule 2: Ubiquitin

Chain B:



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: Ubiquitin/ISG15-conjugating enzyme E2 L6

Chain A:

- Molecule 2: Ubiquitin

Chain B:

5 Refinement protocol and experimental data overview (i)

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

Of the 200 calculated structures, 16 were deposited, based on the following criterion: *Structures with the lowest energy in the lowest energy cluster.*

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

Software name	Classification	Version
HADDOCK	refinement	2.0
HADDOCK	structure solution	2.0
CNS	refinement	1.2

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 16321
Number of chemical shift lists	1
Total number of shifts	1046
Number of shifts mapped to atoms	1039
Number of unparsed shifts	0
Number of shifts with mapping errors	7
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	36%

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	1220	232	1235	9±3
2	B	574	120	599	4±2
All	All	28704	5632	29355	206

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 4.

5 of 74 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:13:LEU:HD21	1:A:101:CYS:HB3	0.80	1.52	2	4
2:B:1:MET:HB2	2:B:63:LYS:HB3	0.66	1.65	6	4
1:A:18:PRO:HG2	1:A:21:LEU:HB3	0.65	1.68	10	2
2:B:19:PRO:HA	2:B:56:LEU:HB2	0.64	1.67	14	2
1:A:27:ASP:HB2	1:A:33:VAL:HB	0.64	1.70	2	2

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	148/152 (97%)	144±1 (97±1%)	4±1 (3±1%)	0±0 (0±0%)	100	100
2	B	71/76 (93%)	70±1 (98±1%)	1±1 (2±1%)	0±0 (0±0%)	100	100
All	All	3504/3648 (96%)	3424 (98%)	80 (2%)	0 (0%)	100	100

There are no Ramachandran outliers.

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	140/143 (98%)	129±3 (92±2%)	11±3 (8±2%)	19	64
2	B	66/69 (96%)	59±2 (89±4%)	7±2 (11±4%)	13	56
All	All	3296/3392 (97%)	3001 (91%)	295 (9%)	16	61

5 of 66 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)
2	B	9	THR	13
1	A	26	SER	12
2	B	22	THR	12
2	B	20	SER	12
2	B	14	THR	12

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 36% for the well-defined parts and 35% for the entire structure.

7.1 Chemical shift list 1

File name: BMRB entry 16321

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	1046
Number of shifts mapped to atoms	1039
Number of unparsed shifts	0
Number of shifts with mapping errors	7
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	1

The following assigned chemical shifts were not mapped to the molecules present in the coordinate file.

- Residue not found in structure. First 5 (of 7) occurences are reported below.

Chain	Res	Type	Atom	Shift Data		
				Value	Uncertainty	Ambiguity
A	97	SER	HB2	3.652	-1.0	2
A	97	SER	HB3	4.009	-1.0	2
A	97	SER	C	175.127	-1.0	1
A	97	SER	N	107.45	-1.0	1
A	97	SER	CB	62.92	-1.0	1

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	144	-0.29 ± 0.12	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	139	-0.18 ± 0.16	None needed (< 0.5 ppm)
$^{13}C'$	127	0.03 ± 0.11	None needed (< 0.5 ppm)

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Nucleus	# values	Correction ± precision, *ppm*	Suggested action
^{15}N	130	0.25 ± 0.41	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 36%, i.e. 1026 atoms were assigned a chemical shift out of a possible 2878. 0 out of 41 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	571/1065 (54%)	179/422 (42%)	264/442 (60%)	128/201 (64%)
Sidechain	455/1636 (28%)	241/964 (25%)	204/595 (34%)	10/77 (13%)
Aromatic	0/177 (0%)	0/93 (0%)	0/74 (0%)	0/10 (0%)
Overall	1026/2878 (36%)	420/1479 (28%)	468/1111 (42%)	138/288 (48%)

7.1.4 Statistically unusual chemical shifts (i)

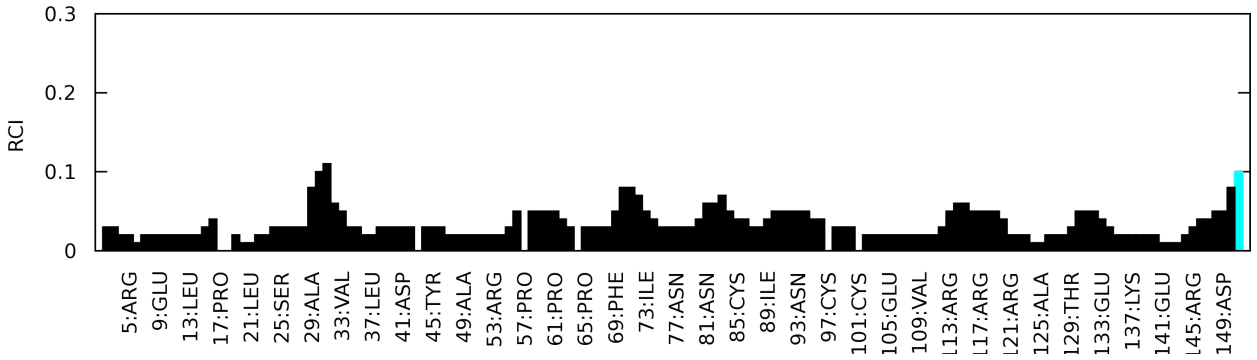
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	77	ASN	H	11.48	11.45 – 5.25	5.1

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:


RCI
0.3
0.2
0.1
0
5:ARG
9:GLU
13:LEU
17:PRO
21:LEU
25:SER
29:ALA
33:VAL
37:LEU
41:ASP
45:TYR
49:ALA
53:ARG
57:PRO
61:PRO
65:PRO
69:PHE
73:ILE
77:ASN
81:ASN
85:CYS
89:ILE
93:ASN
97:CYS
101:CYS
105:GLU
109:VAL
113:ARG
117:ARG
121:ARG
125:ALA
129:THR
133:GLU
137:LYS
141:GLU
145:ARG
149:ASP