wwPDB NMR Structure Validation Summary Report (i)

Apr 21, 2024 – 05:41 PM EDT

PDB ID	:	2KJ3
Title	:	High-resolution structure of the HET-s(218-289) prion in its amyloid form obtained by solid-state NMR
Authors	:	Van Melckebeke, H.; Wasmer, C.; Lange, A.; AB, E.; Loquet, A.; Meier, B.H.
Deposited on	:	2009-05-20

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:

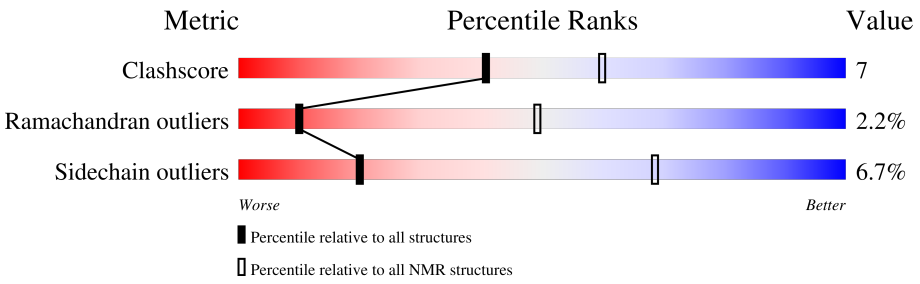
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson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

The overall completeness of chemical shifts assignment was not calculated.

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	79	56% 10% 34%
1	B	79	52% 16% 32%
1	C	79	59% 6% 34%

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, based on the following criterion: *lowest energy*.

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:225-A:249, A:261-A:287, B:225-B:250, B:260-B:287, C:225-C:249, C:261-C:287 (158)	1.18	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 3 clusters and 2 single-model clusters were found.

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

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 3606 atoms, of which 1779 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Small s protein.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	79	1202	371	593	123	114	1	0
1	B	79	1202	371	593	123	114	1	0
1	C	79	1202	371	593	123	114	1	0

There are 21 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	217	MET	-	initiating methionine	UNP Q03689
A	290	HIS	-	expression tag	UNP Q03689
A	291	HIS	-	expression tag	UNP Q03689
A	292	HIS	-	expression tag	UNP Q03689
A	293	HIS	-	expression tag	UNP Q03689
A	294	HIS	-	expression tag	UNP Q03689
A	295	HIS	-	expression tag	UNP Q03689
B	217	MET	-	initiating methionine	UNP Q03689
B	290	HIS	-	expression tag	UNP Q03689
B	291	HIS	-	expression tag	UNP Q03689
B	292	HIS	-	expression tag	UNP Q03689
B	293	HIS	-	expression tag	UNP Q03689
B	294	HIS	-	expression tag	UNP Q03689
B	295	HIS	-	expression tag	UNP Q03689
C	217	MET	-	initiating methionine	UNP Q03689
C	290	HIS	-	expression tag	UNP Q03689
C	291	HIS	-	expression tag	UNP Q03689
C	292	HIS	-	expression tag	UNP Q03689
C	293	HIS	-	expression tag	UNP Q03689
C	294	HIS	-	expression tag	UNP Q03689
C	295	HIS	-	expression tag	UNP Q03689

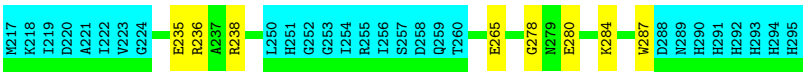
4 Residue-property plots (i)

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.

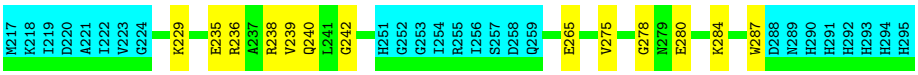
• Molecule 1: Small s protein

Chain A: 56% 10% 34%



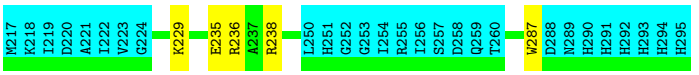
• Molecule 1: Small s protein

Chain B: 52% 16% 32%



• Molecule 1: Small s protein

Chain C: 59% 6% 34%



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: Small s protein

Chain A: 46% 20% 34%

• Molecule 1: Small s protein

Chain B: 43% 25% 32%

M217 K218 I219 D220 A221 I222 V223 G224 R225 N226 S227 A228 K229 T233 E234 E235 R236 A237 R238 V239 Q240 L241 G242 N243 V244 H251 G252 G253 I254 R255 I256 S257 D258 Q259 T260 T261 E265 V275 L276 I277 G278 G285 F286 W287 D288 N289 H290 H291 H292 H293 H294 H295

- Molecule 1: Small s protein

Chain C: 56% 10% 34%

M217 K218 I219 D220 A221 I222 V223 G224 R225 T233 R236 A237 Q240 V244 L250 H251 G252 G253 I254 R255 I256 S257 D258 Q259 T260 L276 W287 D288 N289 H290 H291 H292 H293 H294 H295

5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *simulated annealing, TORSION ANGLE DYNAMICS.*

Of the 200 calculated structures, 20 were deposited, based on the following criterion: *structures with the lowest energy.*

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

Software name	Classification	Version
CYANA	structure calculation	
CNS	refinement	

No chemical shift data was provided. Note: This is a solid-state NMR structure, where hydrogen atoms are typically not assigned a chemical shift value, which may lead to lower completeness of assignment measure.

6 Model quality (i)

6.1 Standard geometry (i)

The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 5$ is considered an outlier worth inspection. RMSZ is the (average) root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	#Z>5	RMSZ	#Z>5
1	A	0.93±0.04	0±0/397 (0.0± 0.0%)	0.72±0.04	0±0/535 (0.0± 0.0%)
1	B	1.02±0.04	0±0/412 (0.0± 0.0%)	0.77±0.04	0±0/556 (0.0± 0.0%)
1	C	0.98±0.03	0±0/397 (0.0± 0.0%)	0.75±0.04	0±0/535 (0.0± 0.0%)
All	All	0.98	0/24120 (0.0%)	0.75	2/32520 (0.0%)

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand. A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	Chirality	Planarity
1	B	0.0±0.0	0.3±0.6
1	A	0.0±0.0	0.4±0.8
1	C	0.0±0.0	0.4±0.7
All	All	0	22

There are no bond-length outliers.

All unique angle outliers are listed below. They are sorted according to the Z-score of the worst occurrence in the ensemble.

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
1	B	232	ARG	CD-NE-CZ	5.51	131.31	123.60	10	1
1	A	225	ARG	NE-CZ-NH1	5.41	123.00	120.30	11	1

There are no chirality outliers.

5 of 11 unique planar outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Group	Models (Total)
1	C	236	ARG	Sidechain	4
1	A	238	ARG	Sidechain	4

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Mol	Chain	Res	Type	Group	Models (Total)
1	B	274	ARG	Sidechain	3
1	C	238	ARG	Sidechain	2
1	B	238	ARG	Sidechain	2

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	393	394	394	7±2
1	B	408	412	412	11±3
1	C	393	394	394	6±2
All	All	23880	24000	24000	357

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 7.

5 of 194 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:239:VAL:HG12	1:A:275:VAL:HB	0.76	1.57	5	2
1:B:239:VAL:HG12	1:B:275:VAL:HB	0.62	1.70	11	4
1:C:244:VAL:HG11	1:C:285:GLY:HA3	0.61	1.73	4	1
1:B:276:LEU:HB3	1:B:287:TRP:CH2	0.60	2.31	13	1
1:B:274:ARG:O	1:C:238:ARG:HA	0.60	1.97	16	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	52/79 (66%)	46±2 (88±3%)	5±1 (10±3%)	1±1 (2±2%)	10 49

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles
1	B	54/79 (68%)	46±1 (86±3%)	6±2 (12±3%)	1±1 (3±2%)	8 44
1	C	52/79 (66%)	46±1 (88±3%)	6±1 (11±3%)	1±1 (2±2%)	12 54
All	All	3160/4740 (67%)	2753 (87%)	337 (11%)	70 (2%)	10 49

5 of 19 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	B	235	GLU	13
1	A	235	GLU	13
1	C	235	GLU	10
1	A	284	LYS	6
1	B	284	LYS	4

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	40/63 (63%)	37±1 (94±3%)	3±1 (6±3%)	21 69
1	B	42/63 (67%)	39±2 (93±4%)	3±2 (7±4%)	19 68
1	C	40/63 (63%)	37±2 (93±4%)	3±2 (7±4%)	19 68
All	All	2440/3780 (65%)	2276 (93%)	164 (7%)	20 68

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	B	236	ARG	14
1	C	236	ARG	11
1	A	238	ARG	11
1	C	229	LYS	11
1	A	280	GLU	9

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)

No chemical shift data were provided