Full wwPDB NMR Structure Validation Report (i)

Jun 12, 2024 – 02:29 AM EDT

PDB ID	:	2KFG
BMRB ID	:	16180
Title	:	Structure of the C-terminal domain of EHD1 in complex with FNYESTDPF-TAK
Authors	:	Kieken, F.; Jovic, M.; Tonelli, M.; Naslavsky, N.; Caplan, S.; Sorgen, P.
Deposited on	:	2009-02-20

This is a Full wwPDB NMR Structure Validation 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:

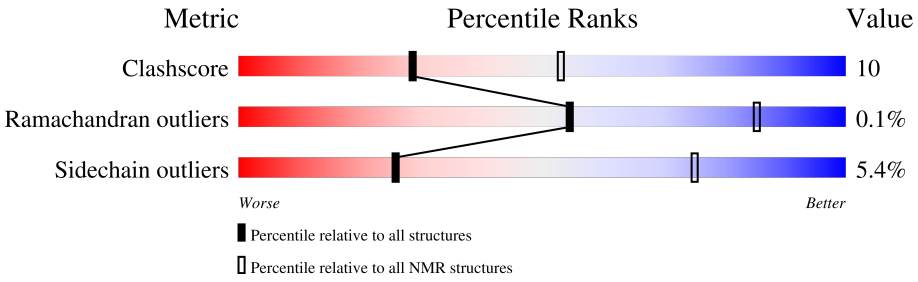
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson 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 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	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	105	64% 19% 17%
2	B	12	33% 67%

2 Ensemble composition and analysis

This entry contains 10 models. Model 8 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:44-A:130, B:149-B:152 (91)	0.61	8

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 and 1 single-model cluster was found.

Cluster number	Models
1	2, 3, 5, 6, 7, 8, 10
2	1, 9
Single-model clusters	4

3 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 1852 atoms, of which 928 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called EH domain-containing protein 1.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	105	1661	527	839	138	156	1	0

There are 5 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	35	GLY	-	expression tag	UNP Q9H4M9
A	36	PRO	-	expression tag	UNP Q9H4M9
A	37	LEU	-	expression tag	UNP Q9H4M9
A	38	GLY	-	expression tag	UNP Q9H4M9
A	39	SER	-	expression tag	UNP Q9H4M9

- Molecule 2 is a protein called Rab11-FIP2 DPF peptide FNYESTDPFTAK.

Mol	Chain	Residues	Atoms					Trace
			Total	C	H	N	O	
2	B	12	190	65	89	14	22	0

- Molecule 3 is CALCIUM ION (three-letter code: CA) (formula: Ca).

Mol	Chain	Residues	Atoms	
			Total	Ca
3	A	1	1	1

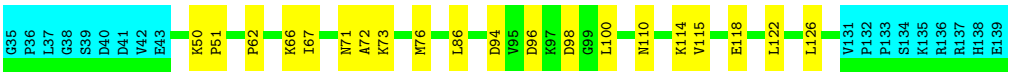
4 Residue-property plots

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.

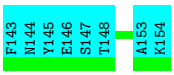
• Molecule 1: EH domain-containing protein 1

Chain A:



• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

Chain B:



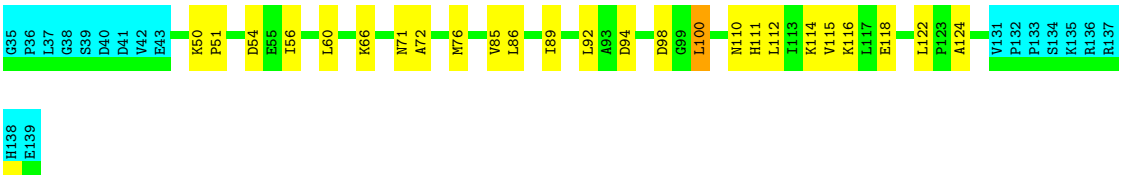
4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

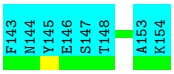
• Molecule 1: EH domain-containing protein 1

Chain A:



• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

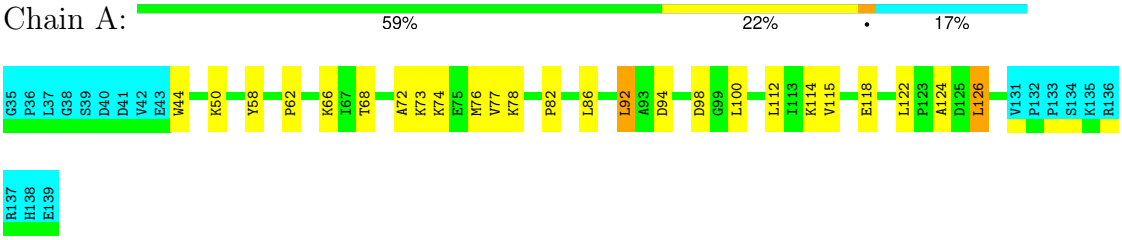
Chain B:



4.2.2 Score per residue for model 2

• Molecule 1: EH domain-containing protein 1

Chain A:



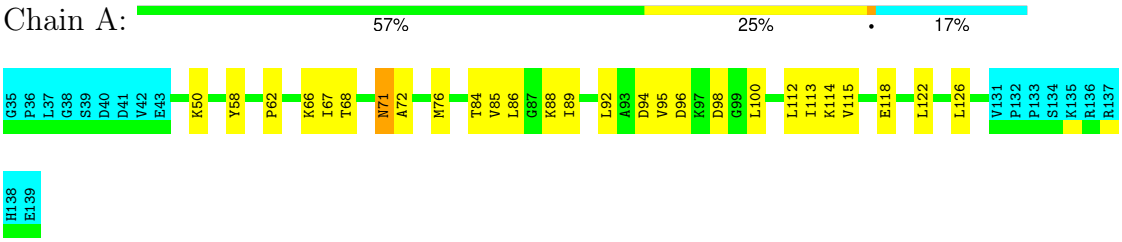
• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

Chain B:

4.2.3 Score per residue for model 3

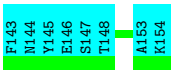
• Molecule 1: EH domain-containing protein 1

Chain A:



• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

Chain B:



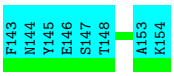
4.2.4 Score per residue for model 4

• Molecule 1: EH domain-containing protein 1

Chain A:

• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

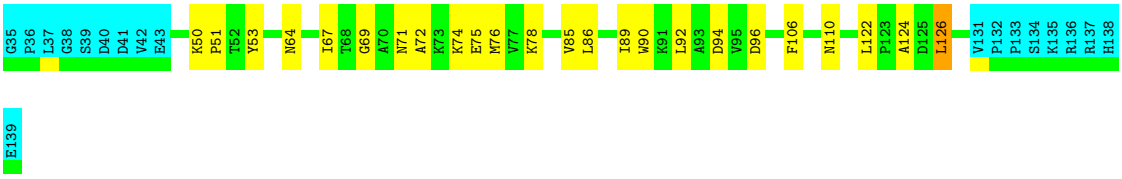
Chain B:



4.2.5 Score per residue for model 5

• Molecule 1: EH domain-containing protein 1

Chain A:



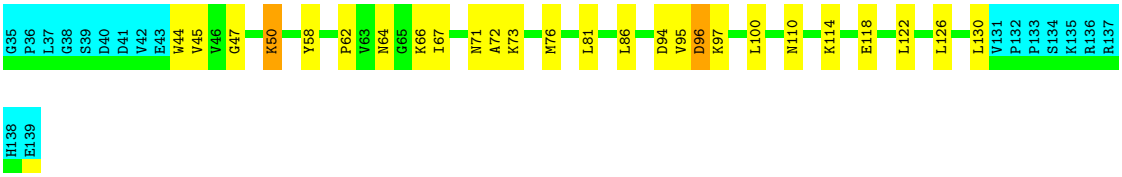
• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

Chain B:

4.2.6 Score per residue for model 6

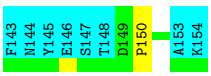
• Molecule 1: EH domain-containing protein 1

Chain A:



• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

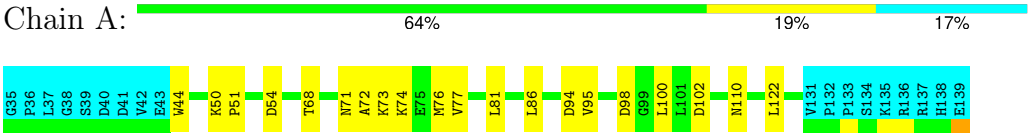
Chain B:



4.2.7 Score per residue for model 7

• Molecule 1: EH domain-containing protein 1

Chain A:



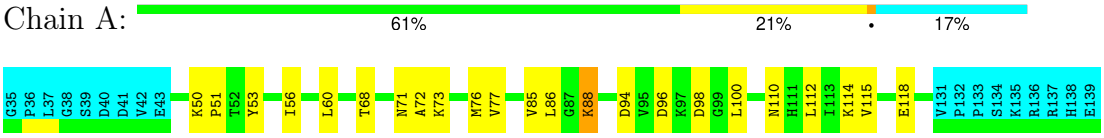
• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

Chain B:

4.2.8 Score per residue for model 8 (medoid)

• Molecule 1: EH domain-containing protein 1

Chain A:



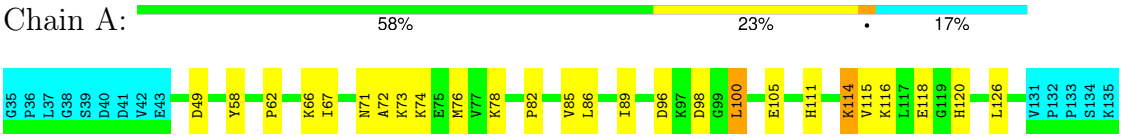
• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

Chain B:

4.2.9 Score per residue for model 9

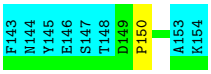
• Molecule 1: EH domain-containing protein 1

Chain A:



• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

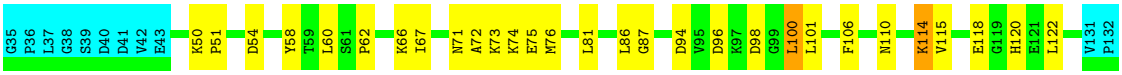
Chain B:

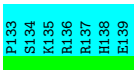


4.2.10 Score per residue for model 10

• Molecule 1: EH domain-containing protein 1

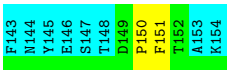
Chain A:





• Molecule 2: Rab11-FIP2 DPF peptide FNYESTDPFTAK

Chain B:



5 Refinement protocol and experimental data overview

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

Of the 30 calculated structures, 10 were deposited, based on the following criterion: *structures with the lowest energy*.

The authors did not provide any information on software used for structure solution, optimization or 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)	working_cs.cif
Number of chemical shift lists	1
Total number of shifts	1273
Number of shifts mapped to atoms	1273
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%

6 Model quality

6.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: CA

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	0.34±0.05	0±1/700 (0.1± 0.1%)	0.44±0.01	0±0/951 (0.0± 0.0%)
2	B	0.46±0.19	0±0/35 (0.0± 0.0%)	0.49±0.04	0±0/49 (0.0± 0.0%)
All	All	0.35	4/7350 (0.1%)	0.45	0/10000 (0.0%)

All unique bond 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(Å)	Ideal(Å)	Models	
								Worst	Total
1	A	53	TYR	CE2-CZ	-6.05	1.30	1.38	5	2
1	A	53	TYR	CE1-CZ	5.47	1.45	1.38	5	2

There are no bond-angle outliers.

There are no chirality outliers.

There are no planarity outliers.

6.2 Too-close contacts

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	683	704	701	15±2
2	B	33	27	27	1±1
3	A	1	0	0	2±0
All	All	7170	7310	7280	151

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 10.

All unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:114:LYS:O	1:A:118:GLU:HG2	0.69	1.86	3	4
1:A:116:LYS:HD3	1:A:122:LEU:HG	0.67	1.66	1	1
1:A:92:LEU:HD11	1:A:124:ALA:HA	0.67	1.67	5	2
1:A:94:ASP:OD1	3:A:141:CA:CA	0.65	1.73	5	9
1:A:96:ASP:OD2	3:A:141:CA:CA	0.65	1.73	5	2
1:A:66:LYS:HB3	1:A:100:LEU:HD11	0.65	1.67	10	1
1:A:98:ASP:OD1	3:A:141:CA:CA	0.65	1.73	2	6
1:A:71:ASN:HA	1:A:74:LYS:HE2	0.64	1.70	7	1
1:A:75:GLU:HA	1:A:78:LYS:HD3	0.64	1.70	5	1
1:A:71:ASN:HA	1:A:74:LYS:HD3	0.64	1.70	9	1
1:A:73:LYS:HD2	2:B:150:PRO:HB2	0.61	1.70	6	5
1:A:68:THR:HA	1:A:100:LEU:HD23	0.61	1.71	2	3
1:A:68:THR:HA	1:A:100:LEU:CD2	0.61	2.25	8	3
1:A:72:ALA:O	1:A:76:MET:HG3	0.60	1.97	2	10
1:A:76:MET:HB3	1:A:86:LEU:HD22	0.59	1.73	6	8
1:A:67:ILE:HB	1:A:71:ASN:OD1	0.58	1.98	6	4
1:A:71:ASN:HA	1:A:74:LYS:CE	0.58	2.28	7	1
1:A:98:ASP:OD2	1:A:100:LEU:HB2	0.58	1.99	4	7
1:A:62:PRO:HA	1:A:67:ILE:HG22	0.57	1.76	4	1
1:A:66:LYS:HD3	1:A:100:LEU:HB3	0.57	1.76	2	3
1:A:114:LYS:O	1:A:118:GLU:HG3	0.56	2.01	6	3
1:A:92:LEU:HD12	1:A:126:LEU:HD23	0.55	1.78	2	1
1:A:112:LEU:O	1:A:115:VAL:HG12	0.55	2.00	3	3
1:A:50:LYS:N	1:A:51:PRO:HD2	0.55	2.17	8	1
1:A:66:LYS:HD3	1:A:100:LEU:CB	0.55	2.32	1	3
1:A:73:LYS:HG3	2:B:151:PHE:CE1	0.54	2.38	2	1
1:A:115:VAL:HG13	1:A:120:HIS:HB2	0.53	1.79	4	2
1:A:92:LEU:HD22	1:A:124:ALA:HA	0.53	1.79	2	1
1:A:74:LYS:O	1:A:78:LYS:HG3	0.53	2.04	2	3
1:A:85:VAL:O	1:A:89:ILE:HG13	0.53	2.04	1	4
1:A:58:TYR:HA	1:A:62:PRO:HG3	0.52	1.80	2	4
1:A:85:VAL:O	1:A:88:LYS:HG2	0.52	2.04	8	1
1:A:74:LYS:HA	1:A:77:VAL:HG12	0.52	1.80	2	1
1:A:96:ASP:HB2	1:A:98:ASP:OD1	0.52	2.03	10	2
1:A:66:LYS:HB2	1:A:100:LEU:HD12	0.50	1.82	9	1
1:A:66:LYS:CD	1:A:100:LEU:HB3	0.50	2.35	3	1
1:A:111:HIS:O	1:A:115:VAL:HG23	0.50	2.07	1	2
1:A:81:LEU:HB2	1:A:86:LEU:HD21	0.49	1.84	10	3

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:56:ILE:O	1:A:60:LEU:HG	0.49	2.07	8	3
1:A:88:LYS:O	1:A:92:LEU:HG	0.49	2.08	3	1
1:A:98:ASP:OD2	1:A:100:LEU:HG	0.49	2.07	10	1
1:A:66:LYS:HB3	1:A:100:LEU:HB3	0.48	1.84	6	1
1:A:100:LEU:HD12	1:A:101:LEU:N	0.48	2.24	10	1
1:A:73:LYS:O	1:A:77:VAL:HG23	0.47	2.09	8	2
1:A:96:ASP:HB3	1:A:105:GLU:OE2	0.47	2.09	9	1
1:A:122:LEU:HD12	1:A:122:LEU:O	0.47	2.09	7	2
1:A:71:ASN:O	1:A:74:LYS:HB3	0.47	2.10	10	1
1:A:45:VAL:HG22	1:A:130:LEU:HD21	0.46	1.88	6	1
1:A:66:LYS:HD3	1:A:100:LEU:HB2	0.45	1.88	1	1
1:A:66:LYS:HD3	1:A:100:LEU:HD21	0.45	1.89	10	1
1:A:76:MET:SD	2:B:151:PHE:CZ	0.45	3.10	7	1
1:A:87:GLY:HA2	2:B:151:PHE:CD2	0.44	2.47	10	1
1:A:58:TYR:HA	1:A:62:PRO:HD3	0.44	1.90	10	1
1:A:67:ILE:HD11	1:A:106:PHE:CD1	0.43	2.47	10	2
1:A:86:LEU:HB3	2:B:151:PHE:CE1	0.43	2.48	5	1
1:A:51:PRO:HA	1:A:54:ASP:HB3	0.43	1.89	10	2
1:A:82:PRO:O	1:A:86:LEU:HG	0.43	2.14	2	2
1:A:68:THR:HA	1:A:100:LEU:HD22	0.42	1.92	8	1
1:A:50:LYS:N	1:A:51:PRO:CD	0.42	2.83	4	4
1:A:69:GLY:HA2	1:A:90:TRP:CH2	0.42	2.50	5	1
1:A:89:ILE:HG23	1:A:112:LEU:HD12	0.42	1.92	1	1
1:A:76:MET:HG2	1:A:113:ILE:HD11	0.41	1.91	3	1
1:A:90:TRP:HB2	2:B:151:PHE:HE2	0.41	1.75	5	1
1:A:116:LYS:HA	1:A:120:HIS:O	0.41	2.15	9	1
1:A:68:THR:HG22	1:A:100:LEU:HD21	0.41	1.92	3	1
1:A:93:ALA:HA	1:A:108:LEU:HD23	0.41	1.93	4	1
1:A:100:LEU:N	1:A:100:LEU:HD12	0.41	2.31	6	1
1:A:60:LEU:HD11	1:A:75:GLU:OE1	0.41	2.16	10	1
1:A:47:GLY:HA2	1:A:50:LYS:HG2	0.40	1.92	6	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	87/105 (83%)	84±1 (96±1%)	3±1 (3±1%)	0±0 (0±0%)	54 85
2	B	4/12 (33%)	4±0 (95±10%)	0±0 (5±10%)	0±0 (0±0%)	100 100
All	All	910/1170 (78%)	877 (96%)	32 (4%)	1 (0%)	54 85

All 1 unique Ramachandran outliers are listed below.

Mol	Chain	Res	Type	Models (Total)
1	A	61	SER	1

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	75/91 (82%)	71±2 (94±3%)	4±2 (6±3%)	24 73
2	B	4/11 (36%)	4±0 (100±0%)	0±0 (0±0%)	100 100
All	All	790/1020 (77%)	747 (95%)	43 (5%)	26 75

All 18 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	110	ASN	7
1	A	126	LEU	5
1	A	50	LYS	4
1	A	122	LEU	4
1	A	71	ASN	3
1	A	100	LEU	3
1	A	44	TRP	3
1	A	96	ASP	2
1	A	64	ASN	2
1	A	114	LYS	2
1	A	54	ASP	1
1	A	92	LEU	1
1	A	84	THR	1
1	A	95	VAL	1
1	A	97	LYS	1
1	A	102	ASP	1

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Mol	Chain	Res	Type	Models (Total)
1	A	88	LYS	1
1	A	49	ASP	1

6.3.3 RNA

There are no RNA molecules in this entry.

6.4 Non-standard residues in protein, DNA, RNA chains

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

6.5 Carbohydrates

There are no monosaccharides in this entry.

6.6 Ligand geometry

Of 1 ligands modelled in this entry, 1 is monoatomic - leaving 0 for Mogul analysis.

6.7 Other polymers

There are no such molecules in this entry.

6.8 Polymer linkage issues

There are no chain breaks in this entry.

7 Chemical shift validation

The completeness of assignment taking into account all chemical shift lists is 87% for the well-defined parts and 80% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *assigned_chem_shift_list_1*

7.1.1 Bookkeeping

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	1273
Number of shifts mapped to atoms	1273
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	8

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	99	1.11 ± 0.17	Should be applied
$^{13}C_{\beta}$	93	1.54 ± 0.26	Should be applied
$^{13}C'$	0	—	None (insufficient data)
^{15}N	86	-0.98 ± 0.42	Should be applied

7.1.3 Completeness of resonance assignments

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. 1085 atoms were assigned a chemical shift out of a possible 1246. 0 out of 19 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	345/447 (77%)	181/181 (100%)	87/182 (48%)	77/84 (92%)
Sidechain	665/703 (95%)	458/458 (100%)	203/229 (89%)	4/16 (25%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	75/96 (78%)	41/47 (87%)	33/41 (80%)	1/8 (12%)
Overall	1085/1246 (87%)	680/686 (99%)	323/452 (71%)	82/108 (76%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 80%, i.e. 1271 atoms were assigned a chemical shift out of a possible 1585. 0 out of 22 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	402/573 (70%)	217/232 (94%)	99/234 (42%)	86/107 (80%)
Sidechain	787/889 (89%)	551/576 (96%)	232/288 (81%)	4/25 (16%)
Aromatic	82/123 (67%)	47/60 (78%)	34/53 (64%)	1/10 (10%)
Overall	1271/1585 (80%)	815/868 (94%)	365/575 (63%)	91/142 (64%)

7.1.4 Statistically unusual chemical shifts (i)

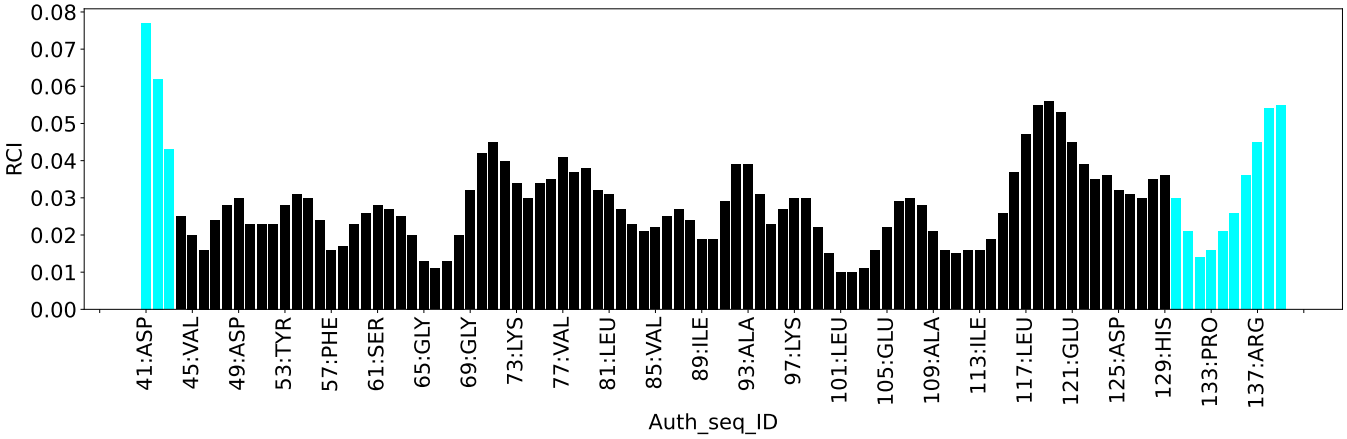
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	59	THR	HG1	6.02	0.08 – 2.19	23.1
1	A	68	THR	HG1	5.40	0.08 – 2.19	20.2
1	A	46	VAL	HB	-1.19	0.43 – 3.54	-10.2
1	A	110	ASN	HD22	3.94	4.69 – 9.61	-6.5
1	A	65	GLY	N	130.59	91.59 – 127.52	5.8
1	A	94	ASP	HB2	1.21	1.41 – 4.01	-5.8
1	A	103	ASP	HA	2.83	3.04 – 6.12	-5.7
1	A	132	PRO	HA	2.70	2.78 – 6.00	-5.2

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:



Random coil index (RCI) for chain B: