Full wwPDB NMR Structure Validation Report (i)

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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:

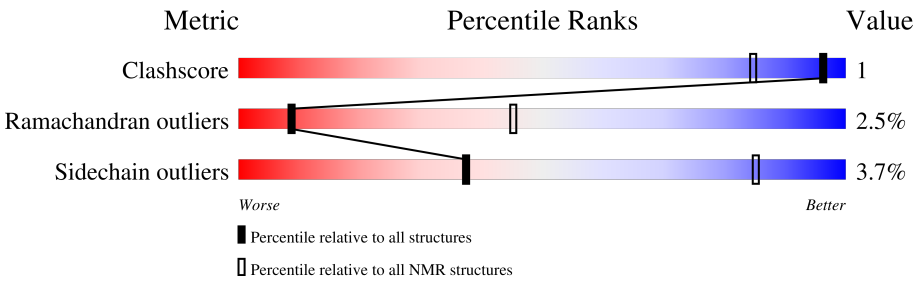
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson 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 26%.

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	L	221	54% 12% • 33%

2 Ensemble composition and analysis (i)

This entry contains 3 models. Model 2 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	L:11-L:21, L:25-L:41, L:46-L:111 (94)	1.30	2
2	L:124-L:128, L:153-L:161, L:182-L:220 (53)	5.40	2

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 1 clusters. No single-model clusters were found.

Cluster number	Models
1	1, 2, 3

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 3226 atoms, of which 1572 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called gelation factor, secretion monitor chimera.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	L	221	3226	1036	1572	283	333	2	0

There are 10 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
L	1	HIS	-	expression tag	UNP P13466
L	2	HIS	-	expression tag	UNP P13466
L	3	HIS	-	expression tag	UNP P13466
L	4	HIS	-	expression tag	UNP P13466
L	5	HIS	-	expression tag	UNP P13466
L	6	HIS	-	expression tag	UNP P13466
L	7	ALA	-	expression tag	UNP P13466
L	8	SER	-	expression tag	UNP P13466
L	203	GLU	-	linker	UNP P62395
L	204	LEU	-	linker	UNP P62395

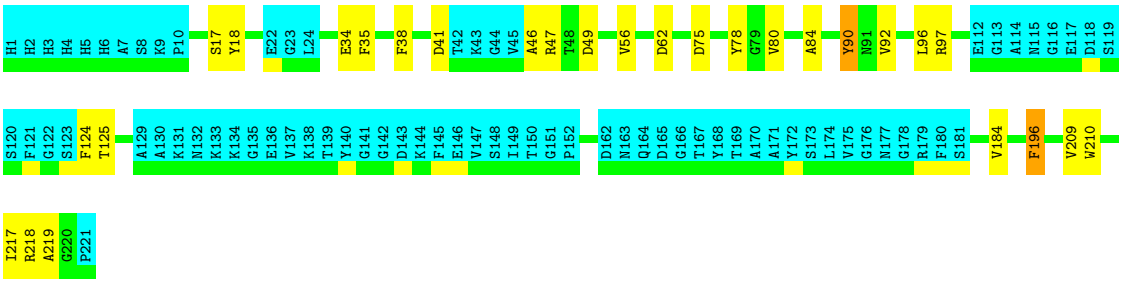
4 Residue-property plots (i)

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: gelation factor, secretion monitor chimera

Chain L: 54% 12% • 33%



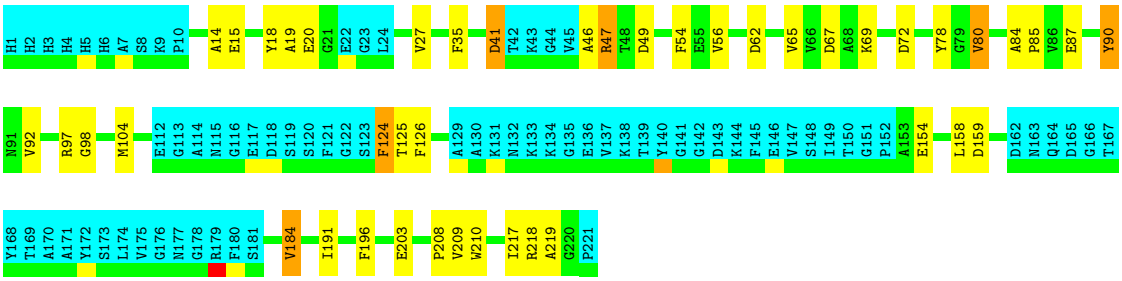
4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

• Molecule 1: gelation factor, secretion monitor chimera

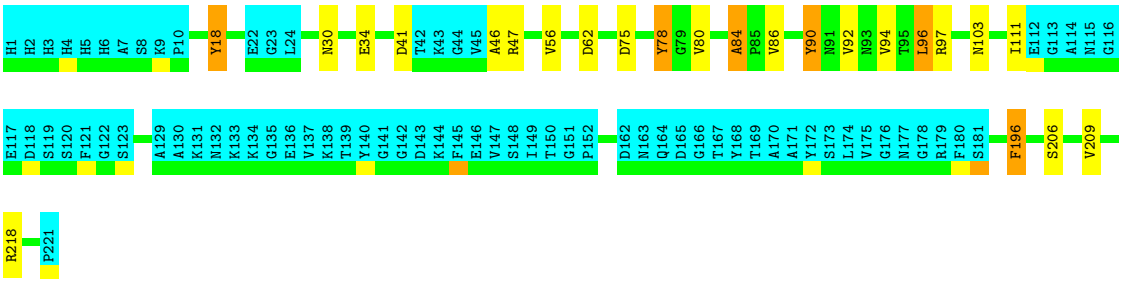
Chain L: 47% 17% • 33%



4.2.2 Score per residue for model 2 (medoid)

- Molecule 1: gelation factor, secretion monitor chimera

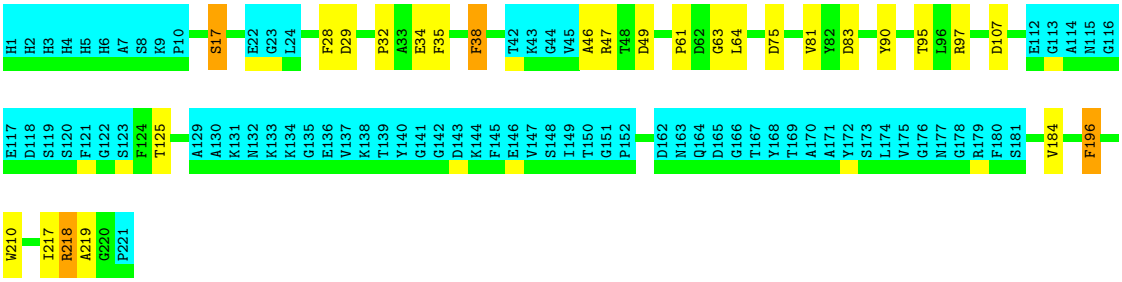
Chain L:



4.2.3 Score per residue for model 3

- Molecule 1: gelation factor, secretion monitor chimera

Chain L:



5 Refinement protocol and experimental data overview (i)

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

Of the 1500 calculated structures, 3 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
GROMACS	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	597
Number of shifts mapped to atoms	597
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	26%

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	L	0.52±0.00	0±0/1129 (0.0± 0.0%)	1.94±0.05	25±6/1544 (1.6± 0.4%)
All	All	0.52	0/3387 (0.0%)	1.94	75/4632 (1.6%)

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	L	0.0±0.0	5.3±1.2
All	All	0	16

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	L	47	ARG	NE-CZ-NH1	14.00	127.30	120.30	1	2
1	L	218	ARG	NE-CZ-NH1	-13.26	113.67	120.30	3	2
1	L	47	ARG	NE-CZ-NH2	-12.99	113.81	120.30	2	3
1	L	218	ARG	NE-CZ-NH2	10.86	125.73	120.30	2	3
1	L	124	PHE	CB-CG-CD1	9.58	127.51	120.80	1	1
1	L	92	VAL	CA-CB-CG1	8.71	123.96	110.90	1	1
1	L	218	ARG	NH1-CZ-NH2	-8.65	109.89	119.40	2	1
1	L	159	ASP	CB-CG-OD1	-8.34	110.79	118.30	1	1
1	L	196	PHE	CB-CG-CD2	-8.28	115.00	120.80	2	1
1	L	61	PRO	N-CA-CB	7.77	112.62	103.30	3	1
1	L	15	GLU	OE1-CD-OE2	-7.51	114.29	123.30	1	1
1	L	184	VAL	CA-CB-CG2	-7.50	99.65	110.90	1	1
1	L	78	TYR	CB-CG-CD1	-7.50	116.50	121.00	2	1
1	L	38	PHE	CB-CG-CD1	-7.49	115.56	120.80	3	1
1	L	78	TYR	CB-CG-CD2	-7.18	116.69	121.00	1	1

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Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)	Models	
								Worst	Total
1	L	97	ARG	NE-CZ-NH2	-6.91	116.84	120.30	2	2
1	L	210	TRP	CG-CD2-CE3	6.89	140.10	133.90	3	1
1	L	85	PRO	N-CA-CB	6.61	111.23	103.30	1	1
1	L	17	SER	CB-CA-C	6.61	122.65	110.10	3	1
1	L	219	ALA	CB-CA-C	6.47	119.81	110.10	3	1
1	L	20	GLU	OE1-CD-OE2	-6.42	115.59	123.30	1	1
1	L	210	TRP	CB-CG-CD2	6.39	134.90	126.60	1	1
1	L	196	PHE	CB-CG-CD1	-6.28	116.40	120.80	3	2
1	L	80	VAL	CA-CB-CG2	6.28	120.32	110.90	1	1
1	L	154	GLU	OE1-CD-OE2	-6.28	115.77	123.30	1	1
1	L	75	ASP	CB-CG-OD1	-6.25	112.67	118.30	3	2
1	L	35	PHE	CB-CG-CD2	-6.22	116.44	120.80	1	1
1	L	65	VAL	CG1-CB-CG2	-6.15	101.06	110.90	1	1
1	L	209	VAL	CA-CB-CG2	6.09	120.03	110.90	2	2
1	L	111	ILE	CA-CB-CG1	6.05	122.49	111.00	2	1
1	L	184	VAL	CA-CB-CG1	6.02	119.93	110.90	3	1
1	L	30	ASN	N-CA-CB	-5.85	100.06	110.60	2	1
1	L	125	THR	CA-CB-CG2	5.79	120.51	112.40	3	1
1	L	107	ASP	CB-CG-OD2	-5.71	113.16	118.30	3	1
1	L	84	ALA	CB-CA-C	5.67	118.61	110.10	2	1
1	L	41	ASP	CB-CG-OD1	-5.65	113.22	118.30	2	1
1	L	78	TYR	CG-CD1-CE1	-5.63	116.80	121.30	2	1
1	L	203	GLU	OE1-CD-OE2	-5.45	116.75	123.30	1	1
1	L	219	ALA	N-CA-CB	-5.45	102.48	110.10	1	1
1	L	46	ALA	CB-CA-C	5.41	118.22	110.10	3	1
1	L	95	THR	C-N-CA	5.40	135.20	121.70	3	1
1	L	54	PHE	CB-CG-CD1	-5.35	117.06	120.80	1	1
1	L	78	TYR	CD1-CE1-CZ	-5.34	114.99	119.80	1	1
1	L	94	VAL	CG1-CB-CG2	-5.34	102.36	110.90	2	1
1	L	210	TRP	CE2-CD2-CG	5.30	111.54	107.30	1	1
1	L	18	TYR	CB-CG-CD2	-5.28	117.83	121.00	2	1
1	L	56	VAL	CA-CB-CG2	5.27	118.81	110.90	2	1
1	L	72	ASP	CB-CG-OD2	5.23	123.01	118.30	1	1
1	L	209	VAL	CG1-CB-CG2	-5.21	102.56	110.90	1	1
1	L	49	ASP	CB-CG-OD1	5.19	122.97	118.30	1	1
1	L	28	PHE	CB-CG-CD2	-5.18	117.17	120.80	3	1
1	L	35	PHE	CB-CG-CD1	-5.18	117.18	120.80	3	1
1	L	97	ARG	NE-CZ-NH1	5.17	122.89	120.30	2	1
1	L	14	ALA	CB-CA-C	5.17	117.86	110.10	1	1
1	L	97	ARG	CD-NE-CZ	5.15	130.81	123.60	3	1
1	L	62	ASP	O-C-N	-5.14	114.46	123.20	2	1

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Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
1	L	67	ASP	CB-CG-OD2	5.14	122.92	118.30	1	1
1	L	210	TRP	CD1-CG-CD2	-5.14	102.19	106.30	1	1
1	L	81	VAL	CA-CB-CG2	5.12	118.59	110.90	3	1
1	L	92	VAL	CG1-CB-CG2	-5.11	102.72	110.90	2	1
1	L	96	LEU	CB-CG-CD1	5.06	119.59	111.00	2	1
1	L	69	LYS	N-CA-CB	5.05	119.69	110.60	1	1
1	L	56	VAL	CA-CB-CG1	5.04	118.46	110.90	1	1
1	L	19	ALA	CB-CA-C	-5.04	102.55	110.10	1	1
1	L	34	GLU	O-C-N	-5.01	114.69	122.70	3	1

There are no chirality outliers.

All 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	L	90	TYR	Sidechain	3
1	L	18	TYR	Peptide,Sidechain	2
1	L	41	ASP	Peptide	1
1	L	47	ARG	Sidechain	1
1	L	124	PHE	Sidechain	1
1	L	125	THR	Peptide	1
1	L	126	PHE	Peptide	1
1	L	78	TYR	Sidechain	1
1	L	96	LEU	Peptide	1
1	L	196	PHE	Sidechain	1
1	L	29	ASP	Sidechain	1
1	L	38	PHE	Sidechain	1
1	L	218	ARG	Sidechain	1

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	L	1104	1066	1066	2±1
All	All	3312	3198	3198	5

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 1.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:L:84:ALA:HB1	1:L:90:TYR:CD1	0.54	2.38	1	2
1:L:34:GLU:HA	1:L:80:VAL:O	0.44	2.13	2	1
1:L:84:ALA:HB1	1:L:90:TYR:CE1	0.42	2.50	2	1
1:L:184:VAL:HG12	1:L:184:VAL:O	0.41	2.15	1	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	L	147/221 (67%)	125±7 (85±5%)	18±5 (12±3%)	4±2 (2±1%)	9	45
All	All	441/663 (67%)	376 (85%)	54 (12%)	11 (2%)	9	45

All 9 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	L	46	ALA	2
1	L	217	ILE	2
1	L	62	ASP	1
1	L	87	GLU	1
1	L	98	GLY	1
1	L	191	ILE	1
1	L	63	GLY	1
1	L	64	LEU	1
1	L	196	PHE	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	L	118/174 (68%)	114±1 (96±1%)	4±1 (4±1%)	37 85
All	All	354/522 (68%)	341 (96%)	13 (4%)	37 85

All 13 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	L	27	VAL	1
1	L	41	ASP	1
1	L	80	VAL	1
1	L	104	MET	1
1	L	158	LEU	1
1	L	208	PRO	1
1	L	86	VAL	1
1	L	103	ASN	1
1	L	206	SER	1
1	L	17	SER	1
1	L	32	PRO	1
1	L	49	ASP	1
1	L	83	ASP	1

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 26% for the well-defined parts and 22% 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 (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	597
Number of shifts mapped to atoms	597
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	0

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	93	-0.48 ± 0.25	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	83	-0.44 ± 0.14	None needed (< 0.5 ppm)
$^{13}C'$	95	0.41 ± 0.18	None needed (< 0.5 ppm)
^{15}N	122	-0.38 ± 0.45	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 26%, i.e. 495 atoms were assigned a chemical shift out of a possible 1881. 0 out of 23 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	418/732 (57%)	162/300 (54%)	169/294 (57%)	87/138 (63%)
Sidechain	77/1007 (8%)	0/657 (0%)	77/320 (24%)	0/30 (0%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	0/142 (0%)	0/70 (0%)	0/69 (0%)	0/3 (0%)
Overall	495/1881 (26%)	162/1027 (16%)	246/683 (36%)	87/171 (51%)

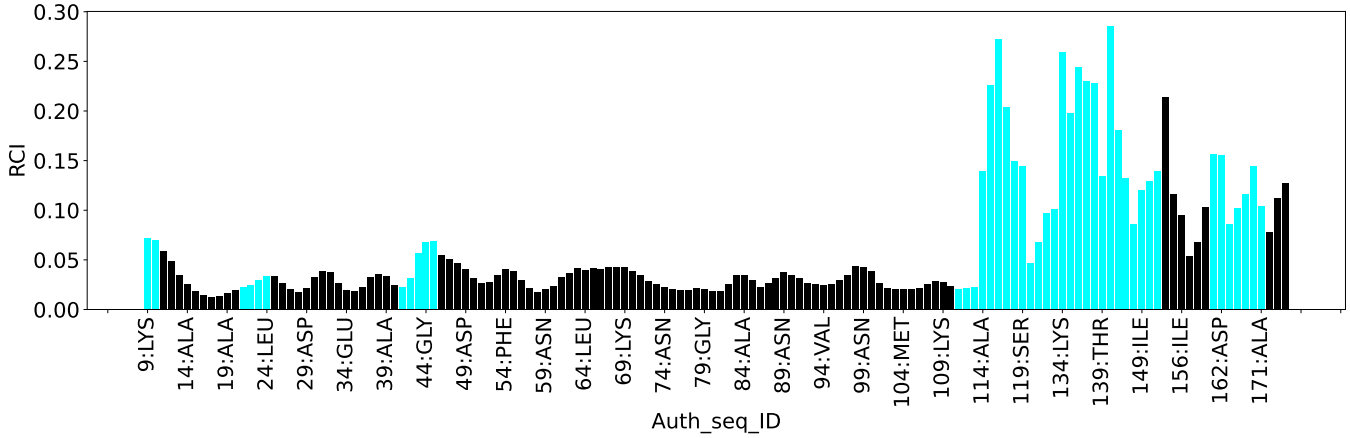
The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 22%, i.e. 597 atoms were assigned a chemical shift out of a possible 2771. 0 out of 29 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	514/1108 (46%)	204/457 (45%)	188/442 (43%)	122/209 (58%)
Sidechain	83/1422 (6%)	0/923 (0%)	83/454 (18%)	0/45 (0%)
Aromatic	0/241 (0%)	0/121 (0%)	0/111 (0%)	0/9 (0%)
Overall	597/2771 (22%)	204/1501 (14%)	271/1007 (27%)	122/263 (46%)

7.1.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

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 L: