Full wwPDB NMR Structure Validation Report (i)

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PDB ID	:	5LKN
Title	:	NMR solution structure of human FNIII domain 2 of NCAM
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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
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:

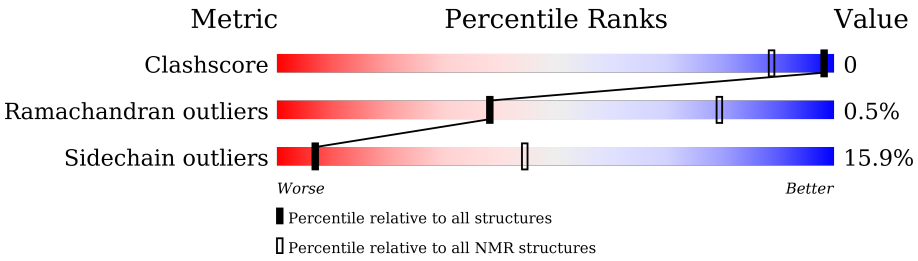
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	unknown
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	rb-20027939
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	rb-20027939

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 81%.

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	103	81% 12% 8%

2 Ensemble composition and analysis

This entry contains 20 models. Model 1 is the overall representative, medoid model (most similar to other models).

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:598-A:692 (95)	0.29	1

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. No single-model clusters were found.

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

3 Entry composition

There is only 1 type of molecule in this entry. The entry contains 1641 atoms, of which 805 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Neural cell adhesion molecule 1.

Mol	Chain	Residues	Atoms						Trace
1	A	103	Total	C	H	N	O	S	0
			1641	523	805	157	153	3	

There are 11 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	595	MET	-	expression tag	UNP P13591
A	596	GLN	-	expression tag	UNP P13591
A	597	PRO	-	expression tag	UNP P13591
A	598	VAL	-	expression tag	UNP P13591
A	599	ARG	-	expression tag	UNP P13591
A	692	HIS	-	expression tag	UNP P13591
A	693	HIS	-	expression tag	UNP P13591
A	694	HIS	-	expression tag	UNP P13591
A	695	HIS	-	expression tag	UNP P13591
A	696	HIS	-	expression tag	UNP P13591
A	697	HIS	-	expression tag	UNP P13591

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: Neural cell adhesion molecule 1

Chain A:

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 (medoid)

• Molecule 1: Neural cell adhesion molecule 1

Chain A:

4.2.2 Score per residue for model 2

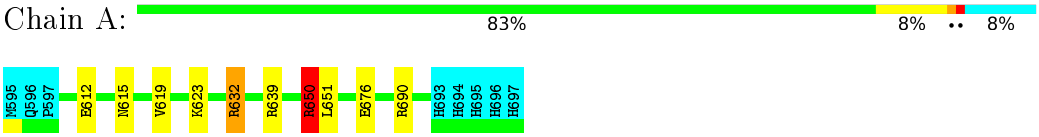
• Molecule 1: Neural cell adhesion molecule 1

Chain A:

4.2.3 Score per residue for model 3

• Molecule 1: Neural cell adhesion molecule 1

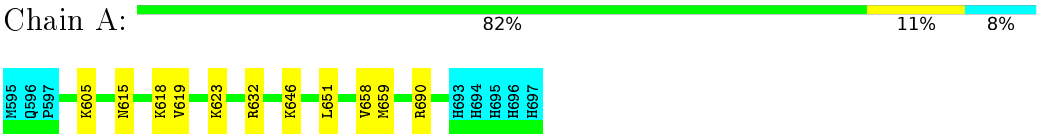
Chain A:



4.2.4 Score per residue for model 4

• Molecule 1: Neural cell adhesion molecule 1

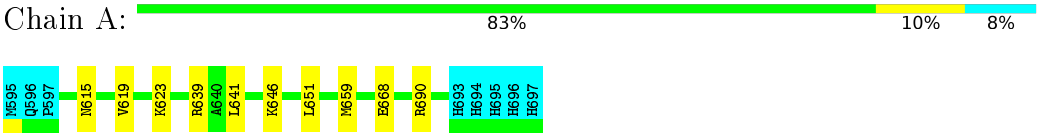
Chain A:



4.2.5 Score per residue for model 5

• Molecule 1: Neural cell adhesion molecule 1

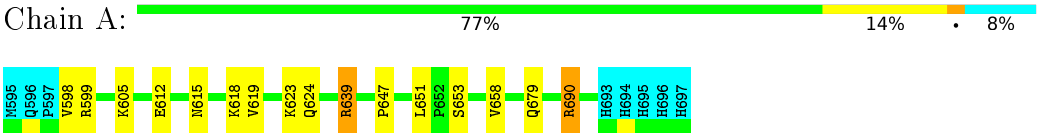
Chain A:



4.2.6 Score per residue for model 6

• Molecule 1: Neural cell adhesion molecule 1

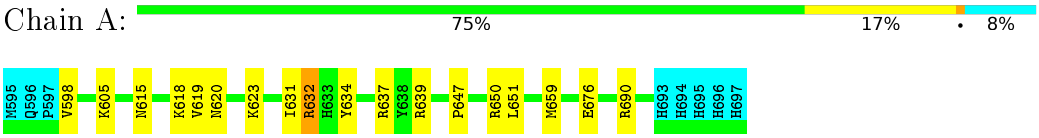
Chain A:



4.2.7 Score per residue for model 7

• Molecule 1: Neural cell adhesion molecule 1

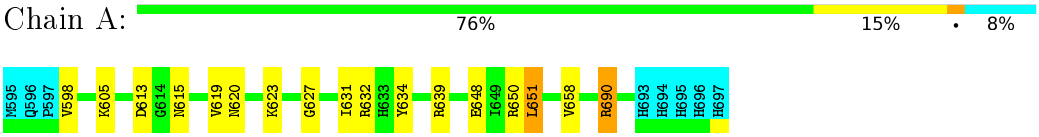
Chain A:



4.2.8 Score per residue for model 8

• Molecule 1: Neural cell adhesion molecule 1

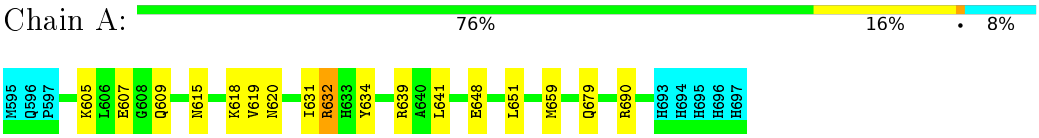
Chain A:



4.2.9 Score per residue for model 9

• Molecule 1: Neural cell adhesion molecule 1

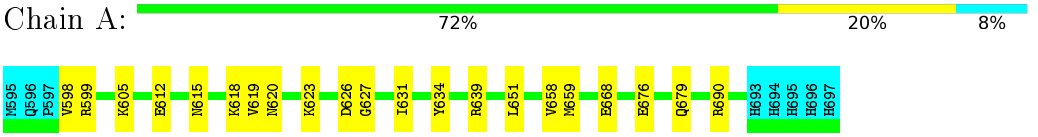
Chain A:



4.2.10 Score per residue for model 10

• Molecule 1: Neural cell adhesion molecule 1

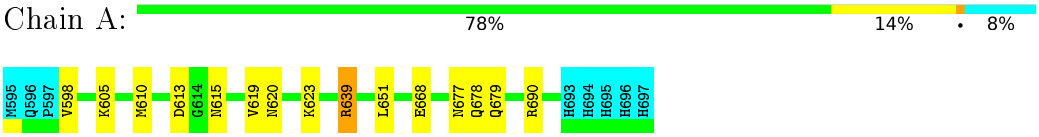
Chain A:



4.2.11 Score per residue for model 11

• Molecule 1: Neural cell adhesion molecule 1

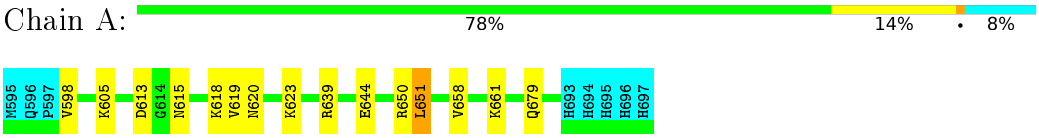
Chain A:



4.2.12 Score per residue for model 12

• Molecule 1: Neural cell adhesion molecule 1

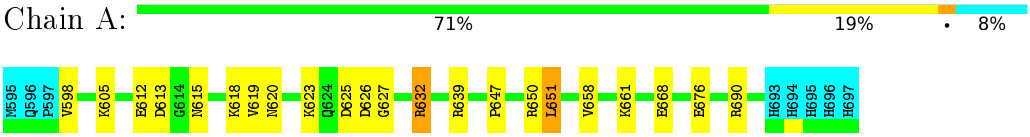
Chain A:



4.2.13 Score per residue for model 13

• Molecule 1: Neural cell adhesion molecule 1

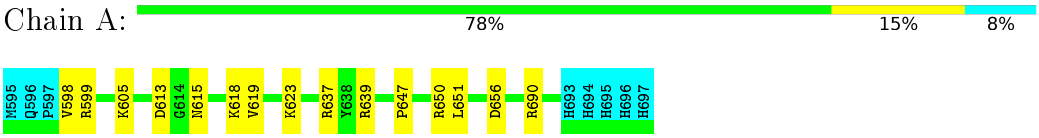
Chain A:



4.2.14 Score per residue for model 14

• Molecule 1: Neural cell adhesion molecule 1

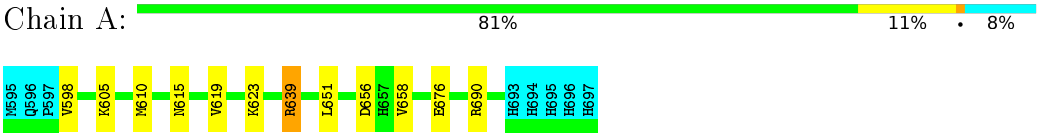
Chain A:



4.2.15 Score per residue for model 15

• Molecule 1: Neural cell adhesion molecule 1

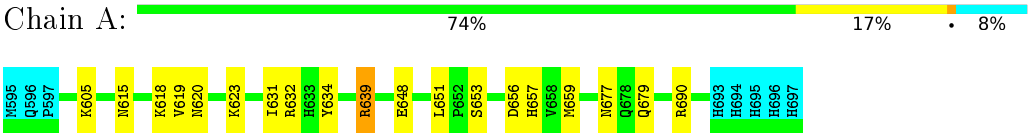
Chain A:



4.2.16 Score per residue for model 16

• Molecule 1: Neural cell adhesion molecule 1

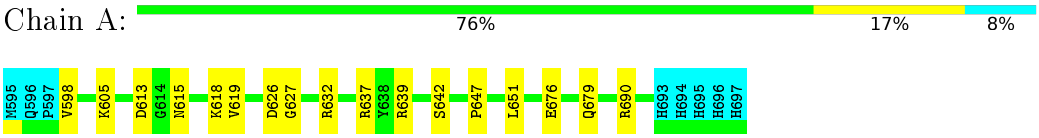
Chain A:



4.2.17 Score per residue for model 17

• Molecule 1: Neural cell adhesion molecule 1

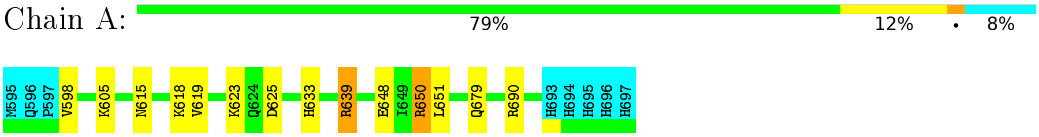
Chain A:



4.2.18 Score per residue for model 18

• Molecule 1: Neural cell adhesion molecule 1

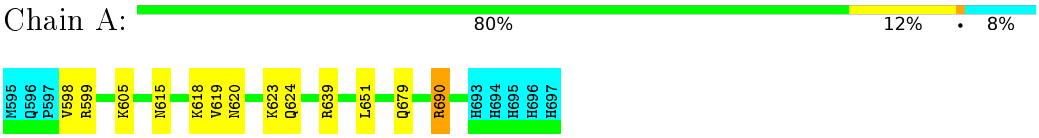
Chain A:



4.2.19 Score per residue for model 19

• Molecule 1: Neural cell adhesion molecule 1

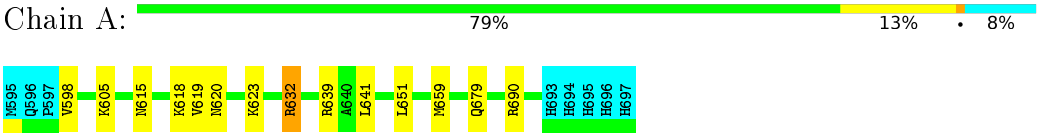
Chain A:



4.2.20 Score per residue for model 20

• Molecule 1: Neural cell adhesion molecule 1

Chain A:



5 Refinement protocol and experimental data overview

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

Of the 100 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
YASARA	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)	5lkn_cs.cif
Number of chemical shift lists	1
Total number of shifts	1106
Number of shifts mapped to atoms	1106
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	81%

No validations of the models with respect to experimental NMR restraints is performed at this time.

6 Model quality

6.1 Standard geometry

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	0.67±0.01	0±0/780 (0.0±0.0%)	0.84±0.02	1±1/1054 (0.1±0.1%)
All	All	0.67	0/15600 (0.0%)	0.84	25/21080 (0.1%)

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	A	690	ARG	NE-CZ-NH1	6.39	123.49	120.30	8	3
1	A	637	ARG	NE-CZ-NH1	6.13	123.36	120.30	17	3
1	A	599	ARG	NE-CZ-NH1	5.97	123.29	120.30	10	4
1	A	632	ARG	NE-CZ-NH1	5.86	123.23	120.30	13	9
1	A	639	ARG	NE-CZ-NH1	5.42	123.01	120.30	16	5
1	A	650	ARG	NE-CZ-NH1	5.17	122.89	120.30	3	1

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	761	744	744	1±1
All	All	15220	14880	14880	14

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 0.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:650:ARG:C	1:A:651:LEU:HD22	0.53	2.24	3	4
1:A:631:ILE:HG21	1:A:634:TYR:CE2	0.49	2.41	8	4
1:A:631:ILE:HG21	1:A:634:TYR:CZ	0.47	2.44	7	1
1:A:650:ARG:O	1:A:651:LEU:HD22	0.43	2.13	1	4
1:A:607:GLU:HG2	1:A:620:ASN:HD22	0.41	1.75	9	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	95/103 (92%)	92±1 (96±1%)	3±2 (3±2%)	1±1 (1±1%)	38	79
All	All	1900/2060 (92%)	1831 (96%)	59 (3%)	10 (1%)	38	79

All 2 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	647	PRO	6
1	A	627	GLY	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	82/90 (91%)	69±3 (84±3%)	13±3 (16±3%)	7	44
All	All	1640/1800 (91%)	1380 (84%)	260 (16%)	7	44

All 36 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	615	ASN	20
1	A	619	VAL	20
1	A	690	ARG	19
1	A	639	ARG	18
1	A	623	LYS	18
1	A	605	LYS	18
1	A	651	LEU	16
1	A	618	LYS	15
1	A	598	VAL	13
1	A	679	GLN	12
1	A	658	VAL	9
1	A	620	ASN	9
1	A	659	MET	8
1	A	613	ASP	7
1	A	676	GLU	6
1	A	632	ARG	5
1	A	648	GLU	4
1	A	612	GLU	4
1	A	656	ASP	4
1	A	668	GLU	4
1	A	626	ASP	3
1	A	641	LEU	3
1	A	661	LYS	3
1	A	677	ASN	3
1	A	610	MET	2
1	A	650	ARG	2
1	A	653	SER	2
1	A	625	ASP	2
1	A	646	LYS	2
1	A	644	GLU	2
1	A	624	GLN	2
1	A	678	GLN	1
1	A	609	GLN	1
1	A	633	HIS	1
1	A	657	HIS	1
1	A	642	SER	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 carbohydrates in this entry.

6.6 Ligand geometry

There are no ligands in this entry.

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 81% for the well-defined parts and 76% for the entire structure.

7.1 Chemical shift list 1

File name: 5lkn_cs.cif

Chemical shift list name: *shift_set_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	1106
Number of shifts mapped to atoms	1106
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	2

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	95	0.35 ± 0.23	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	91	-0.23 ± 0.29	None needed (< 0.5 ppm)
$^{13}C'$	0	—	—
^{15}N	90	0.20 ± 0.50	None needed (< 0.5 ppm)

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 81%, i.e. 965 atoms were assigned a chemical shift out of a possible 1197. 15 out of 15 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	362/465 (78%)	181/185 (98%)	93/190 (49%)	88/90 (98%)
Sidechain	527/630 (84%)	330/373 (88%)	189/224 (84%)	8/33 (24%)

Continued on next page...

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	76/102 (75%)	39/54 (72%)	35/42 (83%)	2/6 (33%)
Overall	965/1197 (81%)	550/612 (90%)	317/456 (70%)	98/129 (76%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 76%, i.e. 1001 atoms were assigned a chemical shift out of a possible 1312. 15 out of 15 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	370/503 (74%)	185/200 (92%)	95/206 (46%)	90/97 (93%)
Sidechain	553/672 (82%)	347/400 (87%)	197/238 (83%)	9/34 (26%)
Aromatic	78/137 (57%)	40/74 (54%)	36/52 (69%)	2/11 (18%)
Overall	1001/1312 (76%)	572/674 (85%)	328/496 (66%)	101/142 (71%)

7.1.4 Statistically unusual chemical shifts (i)

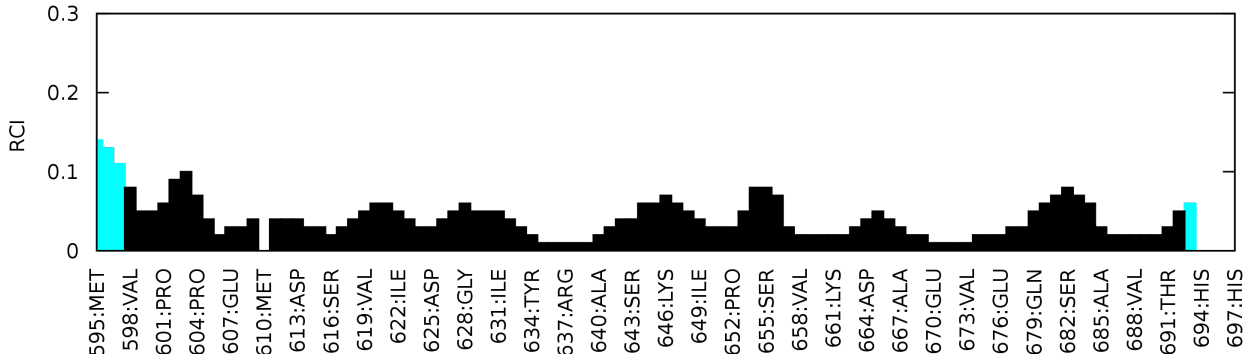
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	639	ARG	HD2	0.46	4.27 – 1.97	-11.6
1	A	615	ASN	HB2	0.61	4.36 – 1.26	-7.1

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
595:MET
598:VAL
601:PRO
604:PRO
607:GLU
510:MET
613:ASP
616:SER
619:VAL
622:ILE
625:ASP
628:GLY
631:ILE
634:TYR
537:ARG
640:ALA
643:SER
646:LYS
649:ILE
652:PRO
655:SER
658:VAL
661:LYS
664:ASP
667:ALA
670:GLU
673:VAL
676:GLU
679:GLN
682:SER
685:ALA
688:VAL
691:THR
694:HIS
697:HIS