wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	2LL4
BMRB ID	:	17667
Title	:	HADDOCK structure of TgMIC4-A5/lacto-N-biose complex, based on NOE-derived distance restraints
Authors	:	Cowper, B.; Matthews, S.
Deposited on	:	2011-10-26

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 (1)) were used in the production of this report:

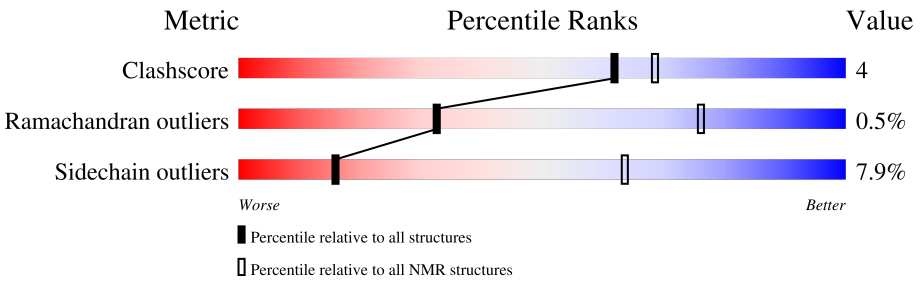
MolProbity	:	4.02b-467
Mogul	:	2022.3.0, CSD as543be (2022)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.37.1

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 90%.

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	M	82	70% 6% 10% 15%
2	A	2	50% 50%

2 Ensemble composition and analysis (i)

This entry contains 10 models. Model 10 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 average*.

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	M:15-M:76 (62)	0.39	10

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 and 6 single-model clusters were found.

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

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 1121 atoms, of which 555 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Micronemal protein 4.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	M	70	1070	330	530	101	102	7	0

- Molecule 2 is an oligosaccharide called beta-D-galactopyranose-(1-3)-2-acetamido-2-deoxy-beta-D-glucopyranose.

Mol	Chain	Residues	Atoms					Trace
			Total	C	H	N	O	
2	A	2	51	14	25	1	11	0

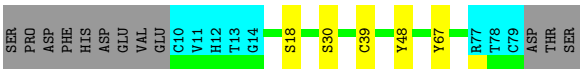
4 Residue-property plots (i)

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: Micronemal protein 4

Chain M:



• Molecule 2: beta-D-galactopyranose-(1-3)-2-acetamido-2-deoxy-beta-D-glucopyranose

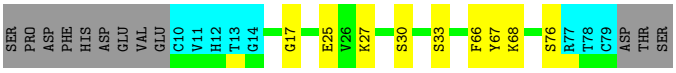
Chain A:

4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 10. Colouring as in section 4.1 above.

• Molecule 1: Micronemal protein 4

Chain M:



• Molecule 2: beta-D-galactopyranose-(1-3)-2-acetamido-2-deoxy-beta-D-glucopyranose

Chain A:

5 Refinement protocol and experimental data overview (i)

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

Of the 200 calculated structures, 10 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
ARIA	refinement	2.1
CNS	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	2
Total number of shifts	855
Number of shifts mapped to atoms	800
Number of unparsed shifts	0
Number of shifts with mapping errors	55
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	90%

6 Model quality (i)

6.1 Standard geometry (i)

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

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	M	482	475	474	4±1
2	A	26	25	24	2±1
All	All	5080	5000	4980	45

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:M:67:TYR:OH	2:A:1:NAG:H1	0.63	1.91	8	4
1:M:67:TYR:CZ	2:A:1:NAG:H5	0.61	2.30	7	7
1:M:39:CYS:SG	1:M:48:TYR:HB3	0.55	2.42	6	5
1:M:67:TYR:CE2	2:A:1:NAG:H1	0.54	2.38	10	4
1:M:67:TYR:CE2	2:A:1:NAG:H5	0.53	2.39	3	3

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	M	62/82 (76%)	56±1 (91±2%)	5±1 (9±2%)	0±0 (0±1%)	32	76
All	All	620/820 (76%)	564 (91%)	53 (9%)	3 (0%)	32	76

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	M	76	SER	2
1	M	54	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	M	52/71 (73%)	48±2 (92±3%)	4±2 (8±3%)	16	63
All	All	520/710 (73%)	479 (92%)	41 (8%)	16	63

5 of 17 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	M	30	SER	6
1	M	39	CYS	4
1	M	33	SER	4
1	M	38	ARG	3
1	M	70	LEU	3

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)

2 monosaccharides are modelled in this entry.

In the following table, the Counts columns list the number of bonds for which Mogul statistics could be retrieved, the number of bonds that are observed in the model and the number of bonds that are defined in the chemical component dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond length is the number of standard deviations the observed value is removed from the expected value. A bond length with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the average root-mean-square of all Z scores of the bond lengths.

Mol	Type	Chain	Res	Link	Bond lengths		
					Counts	RMSZ	#Z>2
2	NAG	A	1	2	15,15,15	0.36±0.01	0±0 (0±0%)
2	GAL	A	2	2	11,11,12	0.21±0.01	0±0 (0±0%)

In the following table, the Counts columns list the number of angles for which Mogul statistics could be retrieved, the number of angles that are observed in the model and the number of angles that are defined in the chemical component dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond angle is the number of standard deviations the observed value is removed from the expected value. A bond angle with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the average root-mean-square of all Z scores of the bond angles.

Mol	Type	Chain	Res	Link	Bond angles		
					Counts	RMSZ	#Z>2
2	NAG	A	1	2	21,21,21	0.66±0.03	0±0 (0±0%)
2	GAL	A	2	2	15,15,17	0.58±0.03	0±0 (0±2%)

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the chemical component dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
2	NAG	A	1	2	-	0±0,6,26,26	0±0,1,1,1
2	GAL	A	2	2	-	0±0,2,19,22	0±0,1,1,1

There are no bond-length outliers.

All unique angle outliers are listed below.

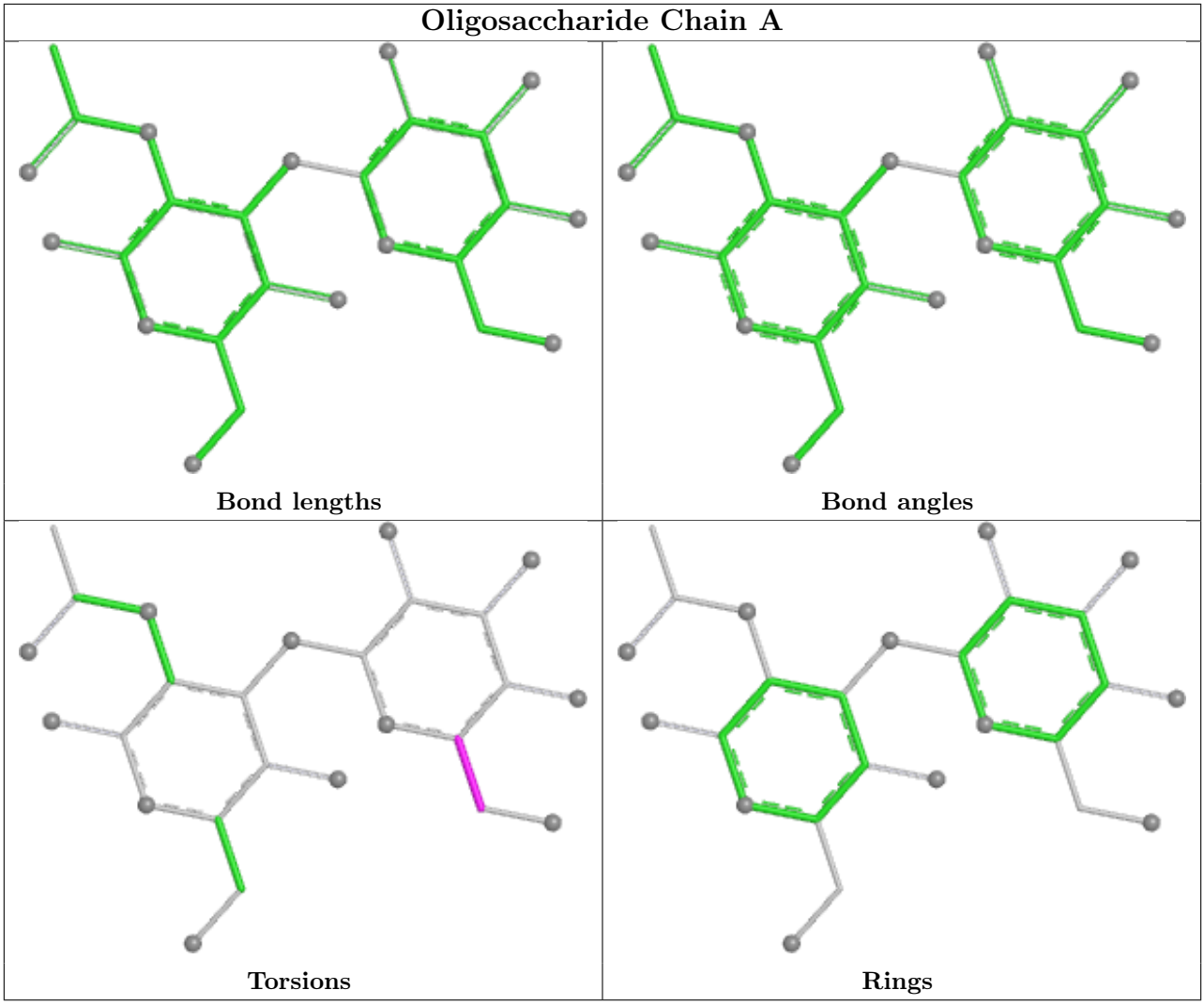
Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
2	A	2	GAL	C2-C3-C4	2.03	107.28	110.86	3	1

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for oligosaccharide.



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 90% for the well-defined parts and 86% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *Protein_shifts*

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	841
Number of shifts mapped to atoms	786
Number of unparsed shifts	0
Number of shifts with mapping errors	55
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	3

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

- No matching atom found in the structure. First 5 (of 55) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	M	5	HIS	C	174.436	.	1
1	M	5	HIS	CA	56.372	.	1
1	M	5	HIS	CB	30.12	.	1
1	M	6	ASP	H	8.157	.	1
1	M	6	ASP	HA	4.56	.	1
1	M	6	ASP	HB2	2.7	.	2
1	M	6	ASP	HB3	2.61	.	2
1	M	6	ASP	C	176.052	.	1
1	M	6	ASP	CA	54.589	.	1
1	M	6	ASP	CB	41.565	.	1
1	M	6	ASP	N	121.137	.	1
1	M	7	GLU	H	8.31	.	1
1	M	7	GLU	CA	56.684	.	1
1	M	7	GLU	CB	30.642	.	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	M	7	GLU	N	120.657	.	1
1	M	8	VAL	H	8.187	.	1
1	M	8	VAL	HA	4.09	.	1
1	M	8	VAL	HB	2.07	.	1
1	M	8	VAL	HG11	0.92	.	2
1	M	8	VAL	HG12	0.92	.	2
1	M	8	VAL	HG13	0.92	.	2
1	M	8	VAL	HG21	0.92	.	2
1	M	8	VAL	HG22	0.92	.	2
1	M	8	VAL	HG23	0.92	.	2
1	M	8	VAL	C	176.033	.	1
1	M	8	VAL	CA	62.418	.	1
1	M	8	VAL	CB	32.728	.	1
1	M	8	VAL	CG1	21.0	.	2
1	M	8	VAL	CG2	21.0	.	2
1	M	8	VAL	N	120.844	.	1
1	M	9	GLU	H	8.479	.	1
1	M	9	GLU	C	175.884	.	1
1	M	9	GLU	CA	57.057	.	1
1	M	9	GLU	CB	29.971	.	1
1	M	9	GLU	CG	35.9	.	1
1	M	9	GLU	N	123.333	.	1
1	M	80	ASP	HA	4.72	.	1
1	M	80	ASP	HB2	2.79	.	2
1	M	80	ASP	HB3	2.65	.	2
1	M	80	ASP	C	176.182	.	1
1	M	80	ASP	CA	54.669	.	1
1	M	80	ASP	CB	41.313	.	1
1	M	81	THR	H	8.09	.	1
1	M	81	THR	HA	4.41	.	1
1	M	81	THR	HB	1.21	.	1
1	M	81	THR	C	173.899	.	1
1	M	81	THR	CA	61.683	.	1
1	M	81	THR	CB	69.667	.	1
1	M	81	THR	CG2	21.35	.	1
1	M	81	THR	N	113.693	.	1
1	M	82	SER	H	7.986	.	1
1	M	82	SER	C	178.749	.	1
1	M	82	SER	CA	60.191	.	1
1	M	82	SER	CB	64.892	.	1
1	M	82	SER	N	123.528	.	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
$^{13}C_{\alpha}$	76	0.10 ± 0.22	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	69	0.07 ± 0.16	None needed (< 0.5 ppm)
$^{13}C'$	74	0.17 ± 0.17	None needed (< 0.5 ppm)
^{15}N	70	-0.77 ± 0.42	None needed (imprecise)

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 90%, i.e. 736 atoms were assigned a chemical shift out of a possible 815. 0 out of 5 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	309/312 (99%)	127/128 (99%)	123/124 (99%)	59/60 (98%)
Sidechain	371/440 (84%)	251/282 (89%)	119/134 (89%)	1/24 (4%)
Aromatic	56/63 (89%)	28/29 (97%)	26/32 (81%)	2/2 (100%)
Overall	736/815 (90%)	406/439 (92%)	268/290 (92%)	62/86 (72%)

7.1.4 Statistically unusual chemical shifts (i)

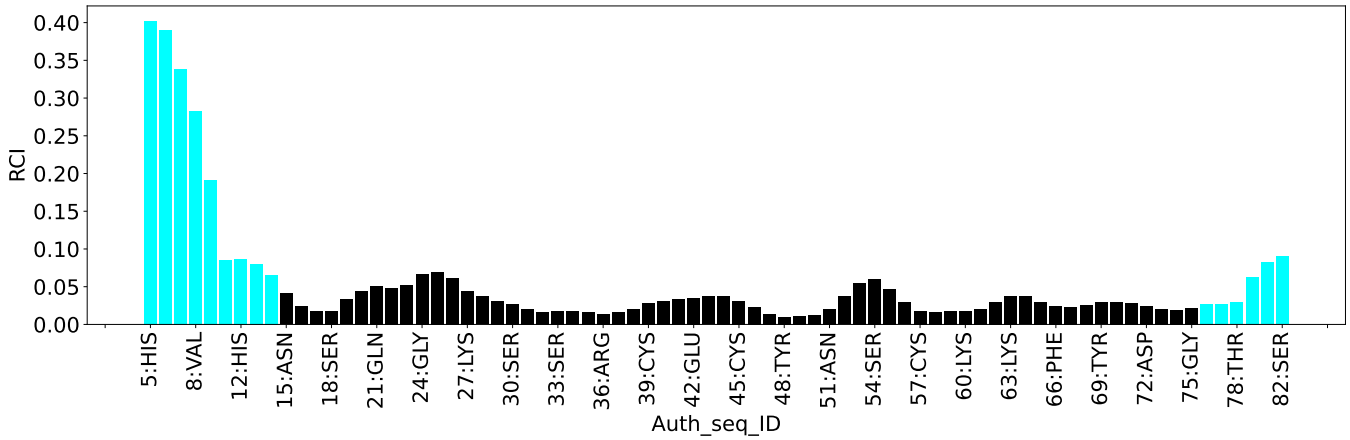
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	M	81	THR	HB	1.21	2.57 – 5.77	-9.2
1	M	60	LYS	HB2	0.03	0.58 – 2.97	-7.3
1	M	64	PRO	HB2	0.36	0.37 – 3.78	-5.0

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 M:



7.2 Chemical shift list 2

File name: working_cs.cif

Chemical shift list name: *Lacto-N-biose_shifts*

7.2.1 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	14
Number of shifts mapped to atoms	14
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

7.2.2 Chemical shift referencing (i)

No chemical shift referencing corrections were calculated (not enough data).

7.2.3 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 0%, i.e. 0 atoms were assigned a chemical shift out of a possible 815. 0 out of 5 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	0/312 (0%)	0/128 (0%)	0/124 (0%)	0/60 (0%)
Sidechain	0/440 (0%)	0/282 (0%)	0/134 (0%)	0/24 (0%)
Aromatic	0/63 (0%)	0/29 (0%)	0/32 (0%)	0/2 (0%)
Overall	0/815 (0%)	0/439 (0%)	0/290 (0%)	0/86 (0%)

7.2.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

7.2.5 Random Coil Index (RCI) plots (i)

No *random coil index*(RCI) plot could be generated from the current chemical shift list. RCI is only applicable to proteins