Full wwPDB NMR Structure Validation Report (i)

Jun 15, 2024 – 12:10 PM EDT

PDB ID	:	2JW1
BMRB ID	:	15504
Title	:	Structural characterization of the type III pilotin-secretin interaction in Shigella flexneri by NMR spectroscopy
Authors	:	Okon, M.S.; Lario, P.I.; Creagh, L.; Jung, Y.M.T.; Maurelli, A.T.; Strynadka, N.C.J.; McIntosh, L.P.
Deposited on	:	2007-10-02

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

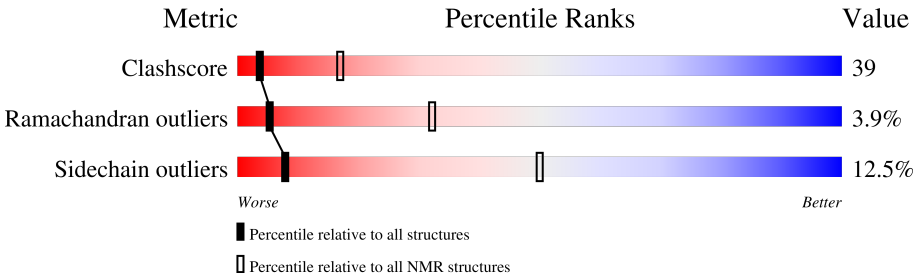
MolProbity	:	4.02b-467
Mogul	:	2022.3.0, CSD as543be (2022)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
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 89%.

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	115	37% 43% 8% 12%
2	B	19	21% 53% 16% 11%

2 Ensemble composition and analysis (i)

This entry contains 15 models. Model 15 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	A:34-A:119, A:125-A:139, B:554-B:570 (118)	0.38	15

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 3 single-model clusters were found.

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

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 2123 atoms, of which 1066 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Lipoprotein mxiM.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	115	1828	582	922	148	171	5	0

- Molecule 2 is a protein called Outer membrane protein mxiD.

Mol	Chain	Residues	Atoms					Trace
			Total	C	H	N	O	
2	B	19	295	95	144	20	36	0

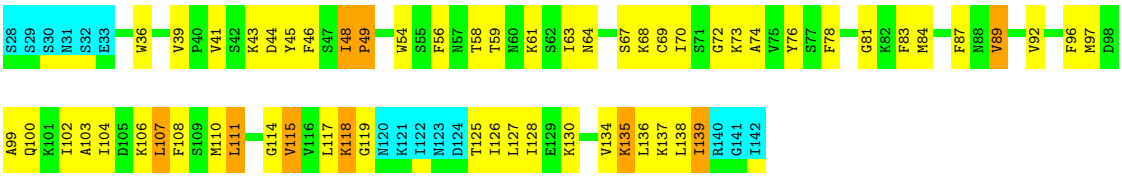
4 Residue-property plots (i)

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.

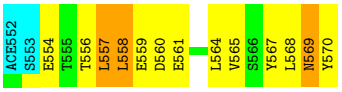
• Molecule 1: Lipoprotein mxiM

Chain A: 37% 43% 8% 12%



• Molecule 2: Outer membrane protein mxiD

Chain B: 21% 53% 16% 11%



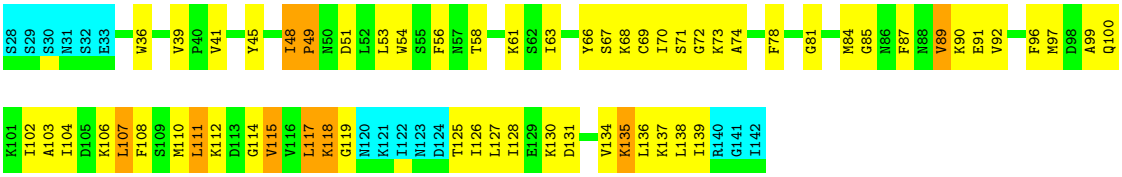
4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

• Molecule 1: Lipoprotein mxiM

Chain A: 35% 45% 8% 12%



• Molecule 2: Outer membrane protein mxiD

Chain B: 21% 58% 11% 11%

ACE552 S553 E554 T555 T556 L557 L558 E559 D560 E561 L564 V565 S566 Y567 L568 N569 Y570

4.2.2 Score per residue for model 2

- Molecule 1: Lipoprotein mxiM

Chain A: 36% 42% 10% 12%

S28 S29 S30 M31 S32 E33 K34 V39 P40 V41 S42 K43 D44 Y45 F46 S47 I48 P49 N50 D51 W54 T58 K61 S62 I63 N64 S67 K68 C69 I70 S71 G72 K73 A74 V75 Y76 S77 F78 G81 K82 F83 M84 G85 N86 F87 N88 V89 K90 E91 V92 F96 A99

Q100 K101 I102 A103 I104 D105 K106 L107 F108 S109 M110 L111 K112 D113 G114 V115 V116 L117 K118 G119 N120 K121 I122 N123 D124 T125 I126 L127 I128 E129 K130 V134 K135 L136 K137 L138 I139 R140 G141 I142

- Molecule 2: Outer membrane protein mxiD

Chain B: 26% 47% 16% 11%

ACE552 S553 E554 T555 T556 L557 L558 E559 D560 E561 L564 V565 S566 Y567 L568 N569 Y570

4.2.3 Score per residue for model 3

- Molecule 1: Lipoprotein mxiM

Chain A: 32% 47% 9% 12%

S28 S29 S30 M31 S32 E33 K34 E35 W36 V39 P40 V41 S42 K43 D44 Y45 F46 S47 I48 P49 W54 S55 F56 N57 T58 T59 N60 K61 S62 I63 N64 S67 K68 C69 I70 S71 G72 K73 A74 V75 Y76 S77 F78 K82 F83 M84 G85 N86 F87 N88 V89 K90 E91 V92 F96

A99 Q100 K101 I102 A103 I104 D105 K106 L107 F108 S109 M110 L111 K112 V115 V116 L117 K118 G119 N120 K121 I122 N123 D124 T125 I126 L127 I128 E129 K130 V134 K135 L136 K137 L138 I139 R140 G141 I142

- Molecule 2: Outer membrane protein mxiD

Chain B: 26% 47% 16% 11%

ACE552 S553 E554 T555 T556 L557 L558 E559 D560 E561 L564 V565 S566 Y567 L568 N569 Y570

4.2.4 Score per residue for model 4

- Molecule 1: Lipoprotein mxiM

Chain A: 37% 38% 12% 12%

S28 S29 S30 M31 S32 E33 W36 V39 P40 V41 S42 K43 D44 Y45 F46 S47 I48 P49 W54 S55 F56 N57 T58 T59 I63 S67 K68 C69 I70 S71 G72 K73 A74 V75 Y76 S77 F78 G81 K82 F83 M84 G85 N86 F87 N88 V89 V92 F96 M97 D98 A99 Q100

K101 I102 A103 I104 D105 K106 L107 F108 S109 M110 L111 V115 V116 L117 K118 G119 N120 K121 I122 N123 D124 T125 I126 L127 I128 E129 K130 D131 V134 K135 L136 K137 L138 I139 R140 G141 I142

- Molecule 2: Outer membrane protein mxiD

Chain B: 16% 53% 21% 11%

AC552 S553 E554 T555 T556 L557 L558 E559 D560 E561 K562 S563 L564 V565 S566 Y567 L568 N569 Y570

4.2.5 Score per residue for model 5

- Molecule 1: Lipoprotein mxiM

Chain A: 41% 37% 10% 12%

S28 S29 S30 M31 S32 E33 W36 V39 P40 V41 S42 K43 D44 Y45 F46 S47 I48 P49 W54 S55 F56 I63 N64 S67 K68 C69 I70 S71 G72 K73 A74 F78 F83 M84 G85 N86 F87 N88 V89 K90 E91 V92 F96 A99 Q100 K101 I102 A103 I104 D105 K106

L107 F108 S109 M110 L111 V115 V116 L117 K118 G119 N120 K121 I122 N123 D124 T125 I126 L127 I128 E129 K130 D131 V134 K135 L136 K137 L138 I139 R140 G141 I142

- Molecule 2: Outer membrane protein mxiD

Chain B: 37% 37% 16% 11%

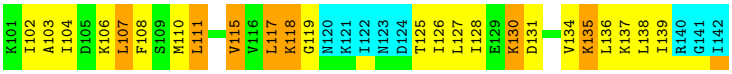
AC552 S553 E554 T555 T556 L557 L558 L564 V565 S566 Y567 L568 N569 Y570

4.2.6 Score per residue for model 6

- Molecule 1: Lipoprotein mxiM

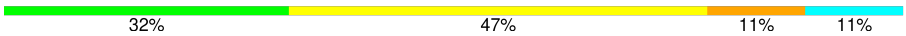
Chain A: 38% 38% 10% • 12%

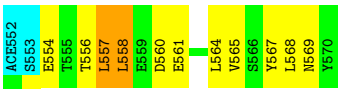
S28 S29 S30 M31 S32 E33 W36 H37 I38 V39 P40 V41 S42 K43 D44 Y45 F46 S47 I48 P49 W54 S55 F56 N57 T58 T59 N60 K61 S62 I63 N64 S67 K68 C69 I70 S71 G72 K73 A74 V75 Y76 S77 F78 M84 F87 N88 V89 V92 F96 M97 D98 A99 Q100



• Molecule 2: Outer membrane protein mxiD

Chain B:

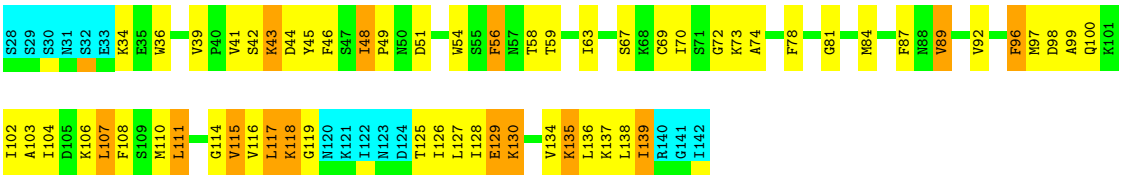




4.2.7 Score per residue for model 7

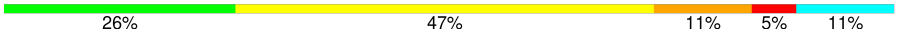
• Molecule 1: Lipoprotein mxiM

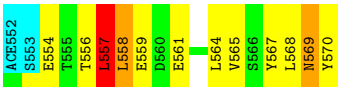
Chain A:



• Molecule 2: Outer membrane protein mxiD

Chain B:

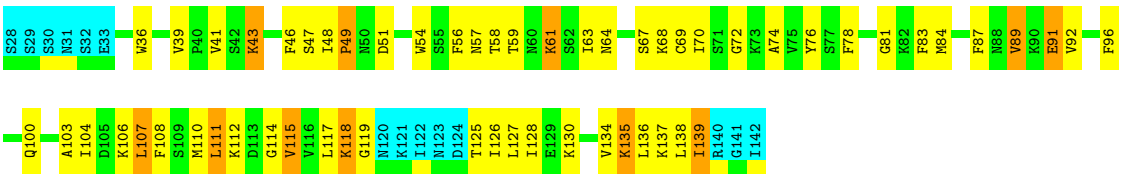




4.2.8 Score per residue for model 8

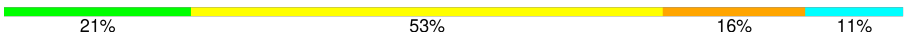
• Molecule 1: Lipoprotein mxiM

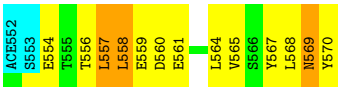
Chain A:



• Molecule 2: Outer membrane protein mxiD

Chain B:

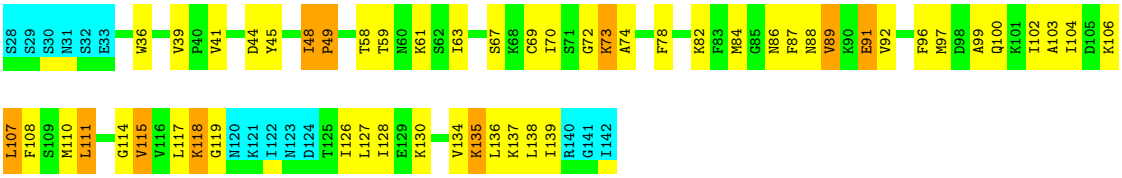




4.2.9 Score per residue for model 9

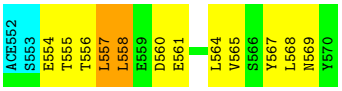
- Molecule 1: Lipoprotein mxiM

Chain A: 42% 37% 9% 12%



- Molecule 2: Outer membrane protein mxiD

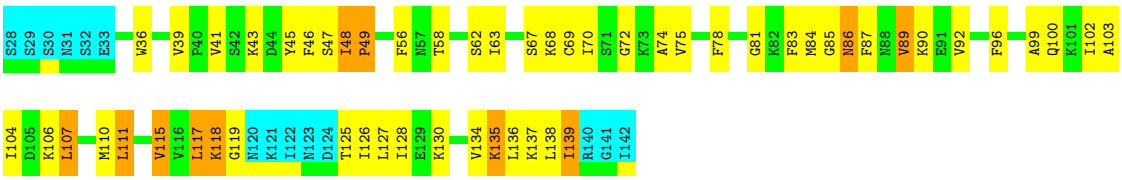
Chain B: 26% 53% 11% 11%



4.2.10 Score per residue for model 10

- Molecule 1: Lipoprotein mxiM

Chain A: 40% 38% 10% 12%



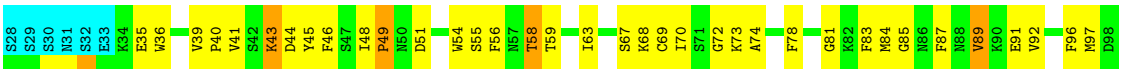
- Molecule 2: Outer membrane protein mxiD

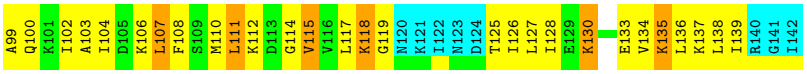
Chain B: 21% 53% 16% 11%

4.2.11 Score per residue for model 11

- Molecule 1: Lipoprotein mxiM

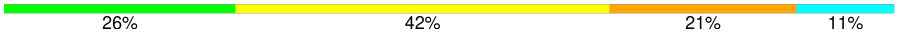
Chain A: 32% 47% 9% 12%

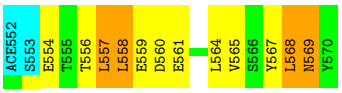




• Molecule 2: Outer membrane protein mxiD

Chain B:

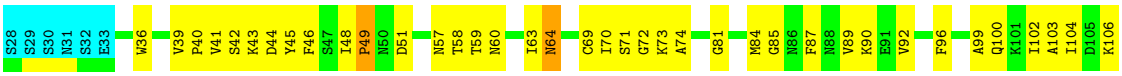


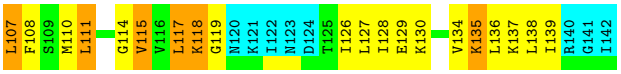


4.2.12 Score per residue for model 12

• Molecule 1: Lipoprotein mxiM

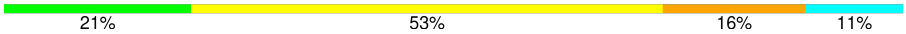
Chain A:

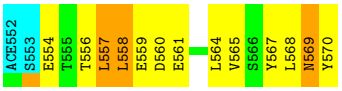




• Molecule 2: Outer membrane protein mxiD

Chain B:

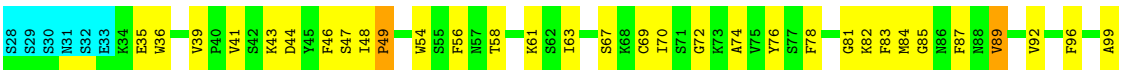


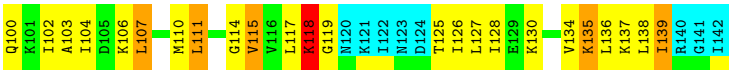


4.2.13 Score per residue for model 13

• Molecule 1: Lipoprotein mxiM

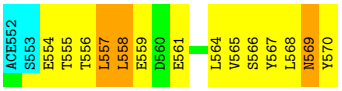
Chain A:





• Molecule 2: Outer membrane protein mxiD

Chain B:



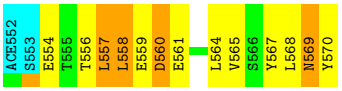
4.2.14 Score per residue for model 14

- Molecule 1: Lipoprotein mxiM

Chain A: 38% 40% 10% 12%

- Molecule 2: Outer membrane protein mxiD

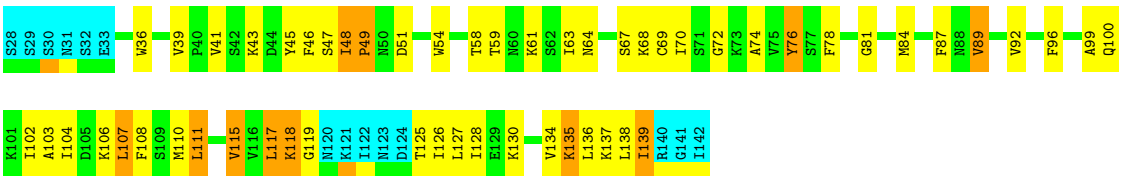
Chain B: 21% 47% 21% 11%



4.2.15 Score per residue for model 15 (medoid)

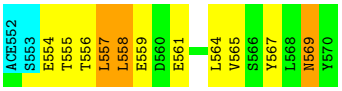
- Molecule 1: Lipoprotein mxiM

Chain A: 40% 38% 10% 12%



- Molecule 2: Outer membrane protein mxiD

Chain B: 32% 42% 16% 11%



5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *simulated annealing, molecular dynamics, torsion angle dynamics.*

Of the 200 calculated structures, 15 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

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	1561
Number of shifts mapped to atoms	1558
Number of unparsed shifts	0
Number of shifts with mapping errors	3
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	89%

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: ACE

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	0.45±0.03	0±0/817 (0.0± 0.0%)	0.57±0.01	0±0/1099 (0.0± 0.0%)
2	B	0.50±0.05	0±0/143 (0.0± 0.0%)	0.65±0.02	0±0/192 (0.0± 0.0%)
All	All	0.46	1/14400 (0.0%)	0.58	0/19365 (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	A	0.0±0.0	0.2±0.4
All	All	0	3

All unique bond outliers are listed below.

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)	Models	
								Worst	Total
1	A	76	TYR	CE1-CZ	6.01	1.46	1.38	6	1

There are no bond-angle outliers.

There are no chirality outliers.

All unique planar outliers are listed below.

Mol	Chain	Res	Type	Group	Models (Total)
1	A	76	TYR	Sidechain	3

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	800	821	820	61±4
2	B	142	136	136	20±3
All	All	14130	14355	14340	1102

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 39.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
2:B:558:LEU:HG	2:B:564:LEU:HA	1.01	1.33	14	15
1:A:48:ILE:HG12	1:A:103:ALA:HB1	0.98	1.33	12	15
1:A:63:ILE:HB	1:A:74:ALA:HB3	0.93	1.38	4	15
1:A:70:ILE:HB	1:A:96:PHE:CZ	0.87	2.05	5	15
1:A:78:PHE:HZ	2:B:564:LEU:HD12	0.84	1.33	5	13
1:A:49:PRO:HG3	1:A:104:ILE:HD11	0.78	1.55	14	14
1:A:127:LEU:HD22	1:A:137:LYS:HD2	0.77	1.57	6	14
1:A:119:GLY:HA3	1:A:127:LEU:HD12	0.76	1.56	14	14
1:A:128:ILE:HB	1:A:136:LEU:HB3	0.76	1.55	12	15
1:A:39:VAL:HG11	1:A:137:LYS:HE2	0.72	1.61	2	13
2:B:558:LEU:HB3	2:B:564:LEU:HG	0.71	1.60	10	15
1:A:58:THR:HG21	2:B:565:VAL:HG12	0.71	1.61	7	9
1:A:54:TRP:HH2	1:A:111:LEU:HG	0.70	1.45	14	12
1:A:126:ILE:HG12	2:B:567:TYR:HE2	0.70	1.46	9	11
1:A:87:PHE:H	1:A:112:LYS:HE2	0.69	1.45	3	2
1:A:63:ILE:HD11	1:A:76:TYR:HB3	0.69	1.64	15	3
1:A:118:LYS:HA	2:B:556:THR:HB	0.69	1.63	9	15
1:A:110:MET:SD	1:A:135:LYS:HG3	0.69	2.28	13	10
1:A:117:LEU:HA	1:A:127:LEU:O	0.69	1.88	13	15
1:A:36:TRP:HB3	1:A:138:LEU:HB3	0.68	1.65	4	12
1:A:78:PHE:CZ	2:B:564:LEU:HD12	0.67	2.22	5	14
1:A:43:LYS:HA	1:A:46:PHE:CD2	0.67	2.25	11	11
2:B:554:GLU:HB2	2:B:557:LEU:HG	0.66	1.65	8	4
2:B:558:LEU:HD11	2:B:567:TYR:CB	0.66	2.20	13	15
1:A:41:VAL:HG21	1:A:134:VAL:HG22	0.65	1.69	8	15
1:A:70:ILE:HB	1:A:96:PHE:HZ	0.64	1.52	10	11

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:81:GLY:HA2	2:B:559:GLU:HB3	0.64	1.68	13	11
1:A:41:VAL:HB	1:A:135:LYS:HA	0.63	1.69	12	7
1:A:49:PRO:HA	1:A:100:GLN:NE2	0.63	2.09	4	7
2:B:558:LEU:HD11	2:B:567:TYR:CG	0.63	2.29	4	15
1:A:85:GLY:O	1:A:112:LYS:HG2	0.63	1.93	11	2
1:A:39:VAL:HB	1:A:137:LYS:HG2	0.63	1.70	2	1
1:A:96:PHE:O	1:A:100:GLN:HB2	0.63	1.93	3	15
1:A:84:MET:HA	1:A:111:LEU:O	0.62	1.94	3	15
1:A:118:LYS:HD3	2:B:554:GLU:HG2	0.62	1.71	4	10
1:A:69:CYS:HB3	1:A:92:VAL:CG2	0.62	2.24	11	15
1:A:48:ILE:HG23	1:A:49:PRO:HD2	0.62	1.72	12	15
1:A:69:CYS:HB3	1:A:92:VAL:HG22	0.62	1.72	8	6
1:A:130:LYS:O	1:A:135:LYS:HE2	0.61	1.96	11	2
1:A:115:VAL:HG11	1:A:128:ILE:HG23	0.60	1.71	2	15
1:A:64:ASN:HA	1:A:72:GLY:O	0.60	1.95	5	3
1:A:41:VAL:HG11	1:A:134:VAL:HG13	0.60	1.74	1	3
2:B:558:LEU:HG	2:B:564:LEU:CA	0.60	2.19	13	7
1:A:117:LEU:HG	1:A:126:ILE:CG2	0.60	2.27	12	4
1:A:72:GLY:HA3	1:A:89:VAL:HG13	0.59	1.73	14	12
1:A:96:PHE:HA	1:A:100:GLN:HE21	0.59	1.56	5	8
1:A:99:ALA:HA	1:A:102:ILE:HD12	0.59	1.73	14	14
1:A:72:GLY:HA3	1:A:89:VAL:HA	0.59	1.73	2	4
1:A:115:VAL:CG1	1:A:128:ILE:HG23	0.59	2.27	3	15
1:A:127:LEU:CD2	1:A:137:LYS:HD2	0.59	2.26	6	3
2:B:558:LEU:CG	2:B:564:LEU:HA	0.58	2.17	13	14
1:A:106:LYS:O	1:A:110:MET:HG3	0.58	1.98	6	14
2:B:557:LEU:O	2:B:558:LEU:HB2	0.58	1.97	15	15
1:A:126:ILE:HB	1:A:138:LEU:HB2	0.58	1.75	11	15
1:A:125:THR:HG23	1:A:139:ILE:HB	0.58	1.76	14	8
2:B:564:LEU:HD22	2:B:568:LEU:HD22	0.58	1.76	11	11
1:A:92:VAL:HG22	1:A:96:PHE:CE1	0.57	2.33	14	7
1:A:49:PRO:HG2	1:A:104:ILE:HD11	0.57	1.73	13	1
1:A:49:PRO:CG	1:A:104:ILE:HD11	0.57	2.29	13	7
1:A:70:ILE:HB	1:A:96:PHE:CE2	0.57	2.34	7	13
2:B:561:GLU:O	2:B:565:VAL:HG13	0.56	2.01	7	14
1:A:72:GLY:CA	1:A:89:VAL:HG13	0.56	2.31	15	5
1:A:67:SER:OG	1:A:70:ILE:HG22	0.55	2.01	14	14
1:A:39:VAL:O	1:A:136:LEU:HA	0.55	2.01	11	14
1:A:45:TYR:CZ	1:A:106:LYS:HB3	0.55	2.36	11	3
1:A:74:ALA:HA	1:A:86:ASN:O	0.55	2.00	4	5
2:B:558:LEU:CB	2:B:564:LEU:HG	0.55	2.32	2	9

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:118:LYS:HG3	2:B:557:LEU:H	0.55	1.61	13	2
1:A:44:ASP:OD2	1:A:106:LYS:HE2	0.55	2.01	14	7
1:A:45:TYR:O	1:A:48:ILE:HG13	0.55	2.02	9	7
1:A:56:PHE:CD1	2:B:568:LEU:HD21	0.54	2.37	6	5
1:A:87:PHE:CE1	1:A:111:LEU:HD23	0.54	2.38	12	6
1:A:42:SER:HB3	1:A:45:TYR:CD1	0.54	2.38	7	1
1:A:87:PHE:CE2	1:A:111:LEU:HD23	0.53	2.37	4	6
1:A:71:SER:HB2	1:A:90:LYS:HE2	0.53	1.80	12	1
1:A:126:ILE:HG12	2:B:567:TYR:CE2	0.53	2.36	9	7
1:A:63:ILE:O	1:A:73:LYS:HA	0.53	2.04	6	3
1:A:117:LEU:O	1:A:118:LYS:HG3	0.53	2.03	12	4
1:A:56:PHE:HE2	1:A:83:PHE:CZ	0.53	2.22	10	5
1:A:51:ASP:HB3	1:A:68:LYS:HE2	0.52	1.82	2	1
1:A:108:PHE:HA	1:A:111:LEU:CD2	0.52	2.35	15	12
1:A:103:ALA:HA	1:A:106:LYS:HD2	0.52	1.80	2	10
1:A:48:ILE:CG2	1:A:49:PRO:HD2	0.52	2.34	3	14
1:A:57:ASN:O	1:A:61:LYS:HA	0.52	2.05	8	1
1:A:92:VAL:HG22	1:A:96:PHE:HE1	0.52	1.65	14	3
1:A:87:PHE:HE2	1:A:111:LEU:HD23	0.52	1.63	14	6
1:A:97:MET:HG3	1:A:100:GLN:OE1	0.51	2.04	14	7
2:B:560:ASP:O	2:B:564:LEU:HB2	0.51	2.04	14	10
1:A:43:LYS:HA	1:A:46:PHE:CD1	0.51	2.40	5	1
1:A:107:LEU:HD13	1:A:136:LEU:HD21	0.51	1.81	2	14
1:A:54:TRP:CH2	1:A:111:LEU:HG	0.51	2.39	2	3
1:A:42:SER:HB3	1:A:45:TYR:HD1	0.51	1.65	7	1
1:A:107:LEU:HD13	1:A:136:LEU:CD2	0.51	2.36	10	13
2:B:558:LEU:HD11	2:B:567:TYR:HB3	0.50	1.82	8	5
1:A:58:THR:CG2	2:B:565:VAL:HG12	0.50	2.37	2	12
1:A:47:SER:O	1:A:100:GLN:HA	0.50	2.06	13	6
1:A:72:GLY:CA	1:A:89:VAL:HA	0.50	2.37	13	7
1:A:119:GLY:HA3	1:A:127:LEU:CD1	0.50	2.36	10	6
1:A:70:ILE:HB	1:A:96:PHE:CE1	0.50	2.40	12	1
1:A:118:LYS:HE2	2:B:554:GLU:HG2	0.50	1.84	13	1
1:A:82:LYS:HD2	1:A:114:GLY:O	0.49	2.07	9	4
1:A:74:ALA:HB2	1:A:87:PHE:CD2	0.49	2.42	7	3
1:A:76:TYR:HA	1:A:84:MET:O	0.49	2.06	13	2
1:A:69:CYS:HB3	1:A:92:VAL:HG21	0.49	1.85	7	5
1:A:116:VAL:HG13	1:A:129:GLU:HB3	0.49	1.85	3	3
1:A:115:VAL:HG21	1:A:128:ILE:CG2	0.48	2.38	6	10
1:A:86:ASN:HA	1:A:112:LYS:HE2	0.48	1.84	2	1
2:B:564:LEU:CD2	2:B:568:LEU:HD22	0.48	2.39	2	3

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:41:VAL:HB	1:A:135:LYS:CA	0.48	2.38	12	2
1:A:74:ALA:HB2	1:A:87:PHE:CD1	0.48	2.44	2	4
1:A:63:ILE:CB	1:A:74:ALA:HB3	0.48	2.26	4	4
1:A:47:SER:O	1:A:100:GLN:HG2	0.48	2.08	15	4
1:A:49:PRO:HD3	1:A:100:GLN:HB3	0.48	1.86	5	2
1:A:36:TRP:CZ3	1:A:139:ILE:HD11	0.48	2.44	5	4
1:A:48:ILE:HG12	1:A:103:ALA:CB	0.47	2.24	12	1
2:B:567:TYR:O	2:B:570:TYR:CD1	0.47	2.68	12	11
1:A:107:LEU:HA	1:A:110:MET:HE2	0.47	1.86	9	1
1:A:45:TYR:CE2	1:A:106:LYS:HB3	0.47	2.44	1	2
1:A:57:ASN:OD1	1:A:59:THR:HB	0.47	2.09	6	1
2:B:566:SER:HA	2:B:569:ASN:ND2	0.47	2.24	13	1
1:A:100:GLN:O	1:A:104:ILE:HG13	0.47	2.10	13	3
1:A:133:GLU:HG3	1:A:135:LYS:HD2	0.46	1.87	11	1
1:A:128:ILE:CB	1:A:136:LEU:HB3	0.46	2.38	15	8
1:A:61:LYS:HG3	1:A:76:TYR:OH	0.46	2.09	15	1
1:A:96:PHE:CD2	1:A:104:ILE:HD12	0.46	2.45	10	6
1:A:48:ILE:CG1	1:A:103:ALA:HB1	0.45	2.41	6	2
1:A:70:ILE:HD11	1:A:89:VAL:HG11	0.45	1.88	15	5
1:A:119:GLY:O	2:B:555:THR:HG21	0.45	2.10	15	2
1:A:110:MET:SD	1:A:135:LYS:HE3	0.45	2.51	11	1
1:A:73:LYS:HG3	1:A:88:ASN:HB2	0.45	1.87	9	1
1:A:39:VAL:CG1	1:A:137:LYS:HE2	0.45	2.41	12	5
1:A:57:ASN:HB3	1:A:60:ASN:OD1	0.45	2.11	12	1
2:B:555:THR:HG23	2:B:556:THR:H	0.45	1.71	15	1
1:A:75:VAL:O	1:A:85:GLY:HA2	0.45	2.12	3	2
1:A:115:VAL:HG23	1:A:130:LYS:CG	0.45	2.42	6	2
1:A:127:LEU:HA	1:A:137:LYS:HB2	0.45	1.88	2	1
1:A:118:LYS:O	1:A:127:LEU:HB2	0.45	2.11	11	4
1:A:73:LYS:HG2	1:A:74:ALA:N	0.45	2.26	2	1
1:A:118:LYS:HD3	2:B:554:GLU:CG	0.45	2.42	7	4
1:A:70:ILE:HG13	1:A:91:GLU:HB3	0.45	1.86	8	2
1:A:107:LEU:O	1:A:110:MET:HB2	0.44	2.11	8	5
1:A:126:ILE:HB	1:A:138:LEU:HD23	0.44	1.89	2	3
1:A:40:PRO:HA	1:A:136:LEU:HD12	0.44	1.90	12	1
1:A:71:SER:HB2	1:A:90:LYS:CG	0.44	2.43	1	1
1:A:63:ILE:CD1	1:A:85:GLY:HA3	0.44	2.42	5	3
1:A:38:ILE:HA	1:A:137:LYS:O	0.43	2.13	6	1
1:A:78:PHE:CE1	1:A:83:PHE:HB2	0.43	2.48	13	1
1:A:108:PHE:HA	1:A:111:LEU:HD22	0.43	1.90	15	1
1:A:117:LEU:HG	1:A:126:ILE:HG22	0.43	1.90	2	2

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:34:LYS:HE2	2:B:568:LEU:O	0.43	2.14	3	1
2:B:565:VAL:O	2:B:569:ASN:ND2	0.43	2.52	15	9
1:A:126:ILE:HA	2:B:556:THR:CG2	0.43	2.44	6	4
1:A:56:PHE:CE2	1:A:83:PHE:CZ	0.43	3.06	5	1
2:B:565:VAL:HA	2:B:568:LEU:HB2	0.43	1.91	11	1
1:A:56:PHE:CZ	1:A:117:LEU:HD21	0.43	2.49	13	1
1:A:119:GLY:H	2:B:555:THR:CG2	0.43	2.26	13	1
1:A:39:VAL:HB	1:A:137:LYS:CG	0.43	2.41	2	1
1:A:111:LEU:HA	1:A:115:VAL:HB	0.43	1.90	13	2
1:A:68:LYS:N	1:A:68:LYS:CD	0.42	2.82	4	1
2:B:566:SER:HA	2:B:569:ASN:HD21	0.42	1.73	13	1
1:A:84:MET:HG2	1:A:114:GLY:HA2	0.42	1.91	8	8
1:A:90:LYS:O	1:A:90:LYS:HG3	0.42	2.13	3	1
1:A:56:PHE:HE1	1:A:83:PHE:CZ	0.42	2.33	11	1
2:B:554:GLU:HG3	2:B:555:THR:HG22	0.42	1.91	13	1
1:A:110:MET:HE1	1:A:136:LEU:HD22	0.42	1.91	4	2
1:A:126:ILE:HA	2:B:556:THR:HG21	0.42	1.91	7	2
1:A:87:PHE:HB3	1:A:89:VAL:CG2	0.42	2.44	13	1
1:A:100:GLN:O	1:A:103:ALA:HB3	0.42	2.14	6	5
2:B:557:LEU:HD23	2:B:557:LEU:HA	0.42	1.71	7	4
1:A:135:LYS:HB3	1:A:135:LYS:NZ	0.41	2.30	1	1
1:A:63:ILE:HD13	1:A:83:PHE:CZ	0.41	2.50	5	1
1:A:42:SER:OG	1:A:135:LYS:HB2	0.41	2.15	12	1
1:A:96:PHE:CD1	1:A:104:ILE:HD12	0.41	2.50	12	1
1:A:43:LYS:HA	1:A:46:PHE:CE2	0.41	2.49	7	1
1:A:130:LYS:O	1:A:131:ASP:HB2	0.41	2.16	5	3
1:A:68:LYS:HD2	1:A:68:LYS:H	0.41	1.75	8	1
1:A:111:LEU:HD13	1:A:111:LEU:N	0.41	2.31	15	2
1:A:110:MET:SD	1:A:135:LYS:CG	0.41	3.09	10	2
1:A:107:LEU:HG	1:A:108:PHE:N	0.41	2.29	1	4
1:A:126:ILE:CG2	1:A:138:LEU:HD23	0.41	2.46	6	1
1:A:92:VAL:HG22	1:A:96:PHE:CE2	0.41	2.50	12	1
1:A:61:LYS:CB	1:A:76:TYR:OH	0.41	2.69	6	1
1:A:82:LYS:HD3	1:A:116:VAL:HB	0.41	1.92	3	1
1:A:36:TRP:CE3	1:A:55:SER:HA	0.41	2.51	11	1
2:B:558:LEU:HD21	2:B:567:TYR:CD2	0.41	2.51	13	1
1:A:87:PHE:HE1	1:A:111:LEU:HD23	0.41	1.73	12	2
1:A:62:SER:HA	1:A:74:ALA:O	0.41	2.16	10	1
1:A:53:LEU:HB2	1:A:66:TYR:O	0.40	2.16	1	1
1:A:96:PHE:C	1:A:100:GLN:HB2	0.40	2.36	15	2
1:A:96:PHE:CD2	1:A:104:ILE:CD1	0.40	3.04	2	1

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:102:ILE:HG22	1:A:106:LYS:HE3	0.40	1.92	7	1
1:A:40:PRO:HB3	1:A:45:TYR:O	0.40	2.16	11	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	101/115 (88%)	89±2 (88±2%)	8±2 (8±2%)	3±1 (3±1%)	6	36
2	B	16/19 (84%)	13±0 (81±2%)	2±0 (12±3%)	1±0 (7±2%)	2	18
All	All	1755/2010 (87%)	1531 (87%)	156 (9%)	68 (4%)	5	32

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	A	118	LYS	15
1	A	139	ILE	15
2	B	558	LEU	15
1	A	49	PRO	13
1	A	51	ASP	6
1	A	97	MET	1
2	B	557	LEU	1
1	A	61	LYS	1
1	A	80	ALA	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	90/103 (87%)	79±2 (88±2%)	11±2 (12±2%)	8	50
2	B	17/18 (94%)	15±1 (86±4%)	2±1 (14±4%)	7	47
All	All	1605/1815 (88%)	1404 (87%)	201 (13%)	8	50

All 33 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	107	LEU	15
1	A	111	LEU	15
1	A	115	VAL	15
1	A	130	LYS	15
1	A	135	LYS	15
2	B	557	LEU	15
2	B	569	ASN	15
1	A	89	VAL	13
1	A	48	ILE	8
1	A	68	LYS	7
1	A	117	LEU	7
1	A	43	LYS	6
1	A	64	ASN	6
1	A	61	LYS	5
1	A	73	LYS	5
1	A	91	GLU	5
1	A	96	PHE	4
1	A	63	ILE	3
1	A	90	LYS	3
1	A	129	GLU	3
1	A	44	ASP	3
1	A	86	ASN	2
2	B	560	ASP	2
2	B	562	LYS	2
1	A	56	PHE	2
1	A	98	ASP	2
1	A	35	GLU	2
1	A	131	ASP	1
1	A	83	PHE	1
1	A	58	THR	1
2	B	568	LEU	1
1	A	118	LYS	1
1	A	93	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 89% 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: *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	1561
Number of shifts mapped to atoms	1558
Number of unparsed shifts	0
Number of shifts with mapping errors	3
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	7

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

- No matching atom found in the structure. All 3 occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	B	19	ACE	H21	2.05	0.02	1
1	B	19	ACE	H22	2.05	0.02	1
1	B	19	ACE	H23	2.05	0.02	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
$^{13}C_{\alpha}$	110	0.09 ± 0.14	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	102	0.11 ± 0.11	None needed (< 0.5 ppm)
$^{13}C'$	104	0.21 ± 0.17	None needed (< 0.5 ppm)
^{15}N	105	0.13 ± 0.55	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 89%, i.e. 1458 atoms were assigned a chemical shift out of a possible 1630. 0 out of 22 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	534/593 (90%)	239/241 (99%)	198/236 (84%)	97/116 (84%)
Sidechain	802/890 (90%)	572/580 (99%)	222/286 (78%)	8/24 (33%)
Aromatic	122/147 (83%)	65/71 (92%)	55/72 (76%)	2/4 (50%)
Overall	1458/1630 (89%)	876/892 (98%)	475/594 (80%)	107/144 (74%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 86%, i.e. 1557 atoms were assigned a chemical shift out of a possible 1810. 0 out of 22 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	579/669 (87%)	260/272 (96%)	214/266 (80%)	105/131 (80%)
Sidechain	856/994 (86%)	608/645 (94%)	239/318 (75%)	9/31 (29%)
Aromatic	122/147 (83%)	65/71 (92%)	55/72 (76%)	2/4 (50%)
Overall	1557/1810 (86%)	933/988 (94%)	508/656 (77%)	116/166 (70%)

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.

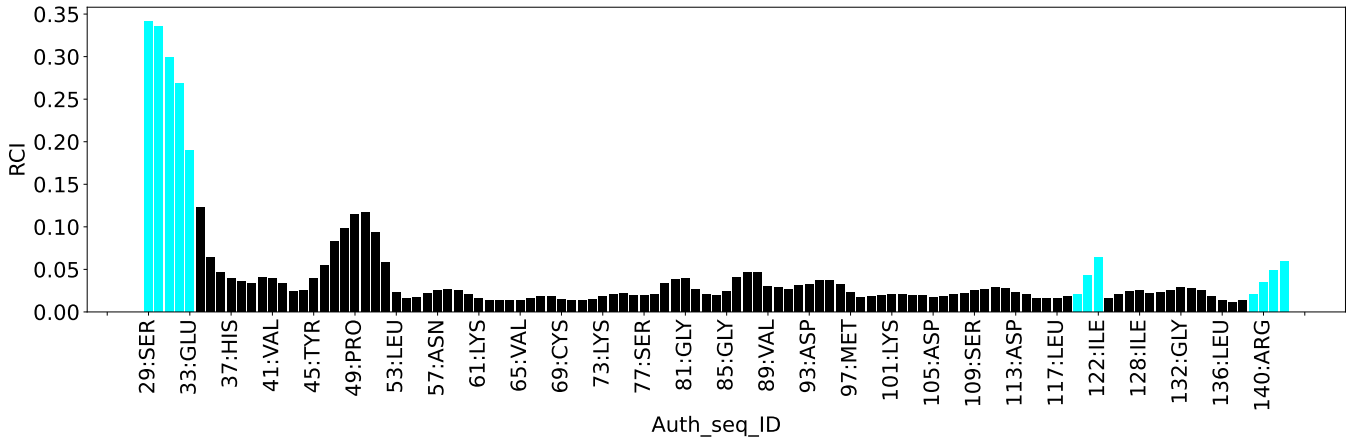
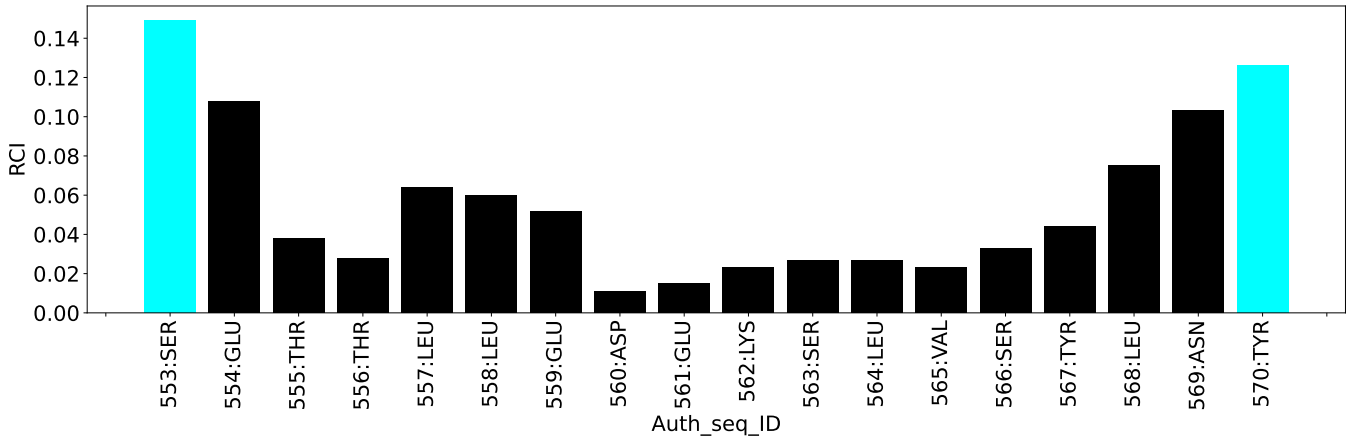
List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	74	ALA	HB1	-0.46	0.14 – 2.58	-7.5
1	A	74	ALA	HB2	-0.46	0.14 – 2.58	-7.5
1	A	74	ALA	HB3	-0.46	0.14 – 2.58	-7.5
1	A	54	TRP	HE1	5.53	6.88 – 13.28	-7.1
1	A	138	LEU	HD21	-1.00	-0.65 – 2.13	-6.2
1	A	138	LEU	HD22	-1.00	-0.65 – 2.13	-6.2
1	A	138	LEU	HD23	-1.00	-0.65 – 2.13	-6.2

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: