Full wwPDB NMR Structure Validation Report (i)

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PDB ID	:	6TR0
BMRB ID	:	34466
Title	:	Solution structure of U2AF2 RRM1,2
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Deposited on	:	2019-12-17

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:

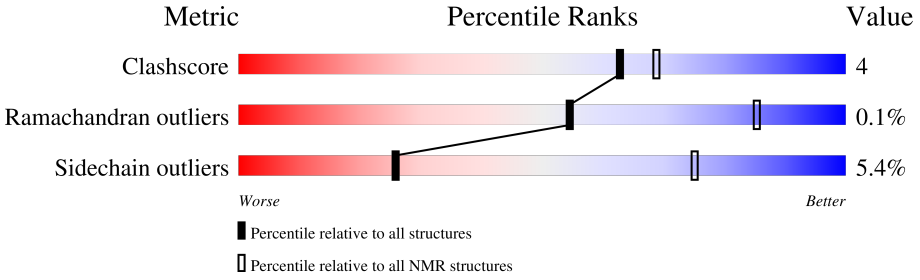
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
BMRB Restraints Analysis	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.33

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 84%.

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	205	72% 9% 20%

2 Ensemble composition and analysis (i)

This entry contains 10 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	A:145-A:192, A:197-A:227 (79)	0.49	10
2	A:250-A:335 (86)	0.54	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 2 clusters. No single-model clusters were found.

Cluster number	Models
1	1, 2, 3, 4, 6, 7, 9
2	5, 8, 10

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 3137 atoms, of which 1568 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Splicing factor U2AF 65 kDa subunit.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	205	3137	995	1568	269	296	9	0

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	138	ALA	-	expression tag	UNP P26368
A	139	MET	-	expression tag	UNP P26368

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: Splicing factor U2AF 65 kDa subunit

Chain A: 72% 9% 20%

A138 M139 V140 G141 S142 Q143 M144 F168 A171 Q172 M173 R174 L175 T179 Q193 D194 K195 N196 L200 E201 F202 T208 F219 Q222 R228 P229 H230 D231 Y232 Q233 P234 L235 P236 G237 M238 S239 E240 N241 P242 S243 V244 Y245 V246 P247 G248 V249 D272 K276

T280 G283 Q324 L325 R334 A335 S336 V337 G338 A339 K340 N341 A342

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: Splicing factor U2AF 65 kDa subunit

Chain A: 70% 10% 20%

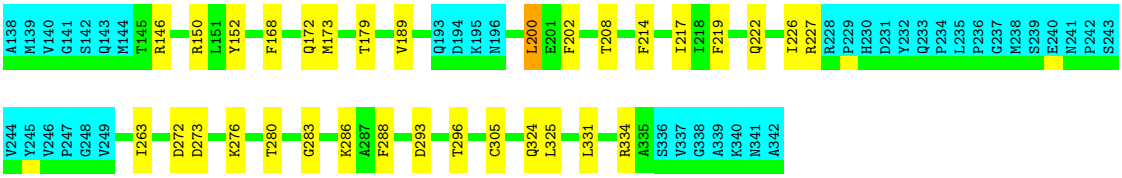
A138 M139 V140 G141 S142 Q143 M144 N155 F168 A171 Q172 M173 R174 L175 T179 Q193 D194 K195 N196 F202 T208 F219 Q222 S223 R228 P229 H230 D231 Y232 Q233 P234 L235 P236 G237 M238 S239 E240 N241 P242 S243 V244 Y245 V246 P247 G248 V249 V250 Q274

T280 G283 F288 C305 N311 Q324 Q333 S336 V337 G338 A339 K340 N341 A342

4.2.2 Score per residue for model 2 (medoid)

• Molecule 1: Splicing factor U2AF 65 kDa subunit

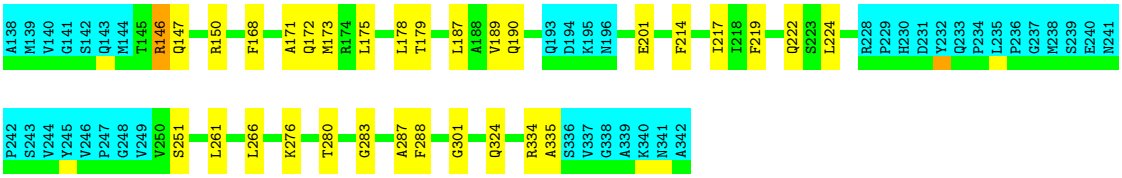
Chain A: 65% 15% 20%



4.2.3 Score per residue for model 3

- Molecule 1: Splicing factor U2AF 65 kDa subunit

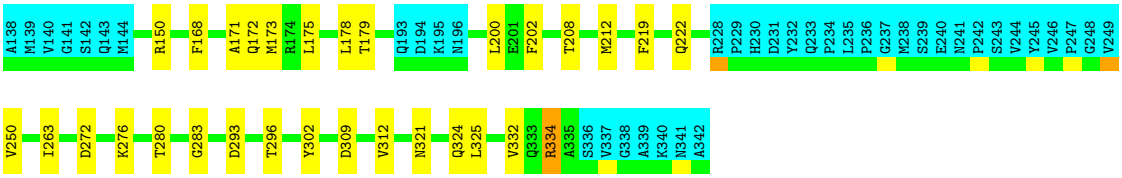
Chain A:



4.2.4 Score per residue for model 4

- Molecule 1: Splicing factor U2AF 65 kDa subunit

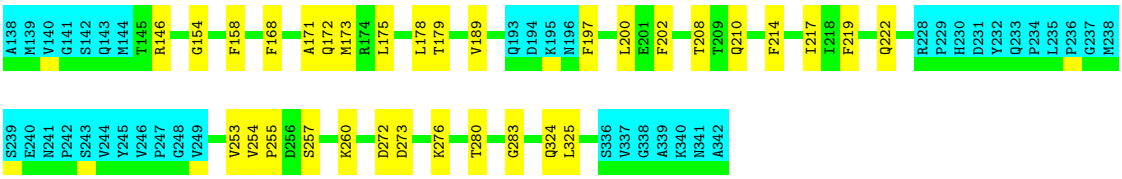
Chain A:



4.2.5 Score per residue for model 5

- Molecule 1: Splicing factor U2AF 65 kDa subunit

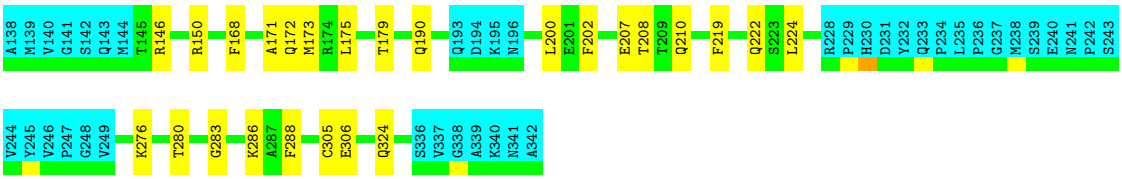
Chain A:



4.2.6 Score per residue for model 6

- Molecule 1: Splicing factor U2AF 65 kDa subunit

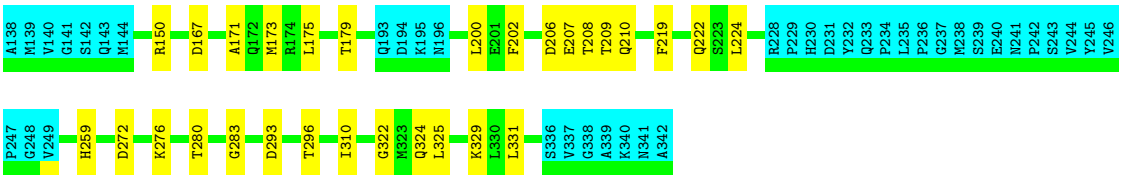
Chain A: 68% 12% 20%



4.2.7 Score per residue for model 7

- Molecule 1: Splicing factor U2AF 65 kDa subunit

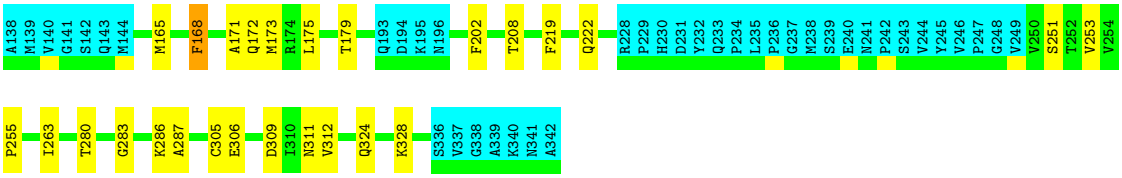
Chain A: 66% 14% 20%



4.2.8 Score per residue for model 8

- Molecule 1: Splicing factor U2AF 65 kDa subunit

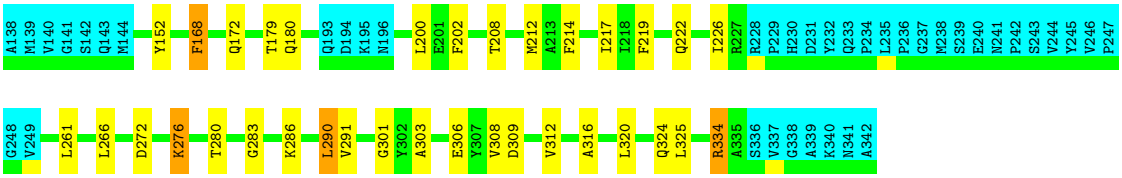
Chain A: 68% 12% 20%



4.2.9 Score per residue for model 9

- Molecule 1: Splicing factor U2AF 65 kDa subunit

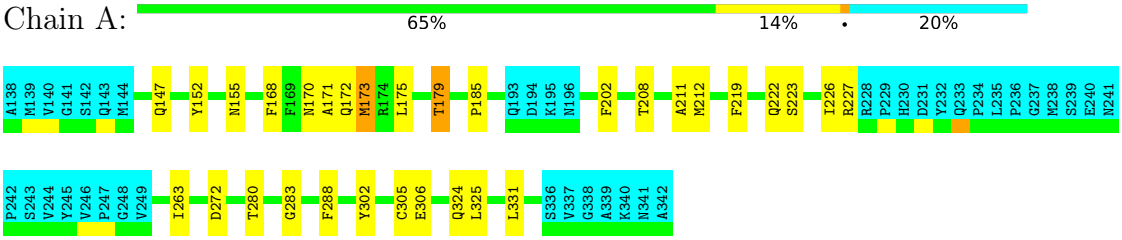
Chain A: 64% 15% • 20%



4.2.10 Score per residue for model 10

- Molecule 1: Splicing factor U2AF 65 kDa subunit

Chain A:



5 Refinement protocol and experimental data overview (i)

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

Of the 250 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
CYANA	structure calculation	
ARIA	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	2243
Number of shifts mapped to atoms	2243
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	84%

6 Model quality (i)

6.1 Standard geometry (i)

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	A	1272	1280	1279	11±2
All	All	12720	12800	12790	113

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.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:219:PHE:O	1:A:222:GLN:HG2	0.64	1.92	9	10
1:A:272:ASP:O	1:A:276:LYS:HB2	0.63	1.94	9	2
1:A:280:THR:HA	1:A:283:GLY:O	0.60	1.97	3	10
1:A:171:ALA:O	1:A:175:LEU:HG	0.55	2.01	10	8
1:A:168:PHE:O	1:A:172:GLN:HG2	0.54	2.03	3	8
1:A:286:LYS:HB2	1:A:308:VAL:HG22	0.54	1.78	9	1
1:A:202:PHE:HB2	1:A:208:THR:OG1	0.54	2.03	2	9
1:A:276:LYS:HB2	1:A:288:PHE:CD1	0.53	2.38	3	1
1:A:290:LEU:HD13	1:A:291:VAL:N	0.53	2.18	9	1
1:A:259:HIS:CE1	1:A:310:ILE:HB	0.53	2.39	7	1
1:A:309:ASP:HB3	1:A:311:ASN:ND2	0.52	2.20	8	1
1:A:272:ASP:O	1:A:276:LYS:HG3	0.50	2.06	2	3
1:A:207:GLU:HA	1:A:210:GLN:CD	0.50	2.28	7	2
1:A:150:ARG:HA	1:A:200:LEU:O	0.49	2.07	2	4

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:154:GLY:HA2	1:A:197:PHE:CD1	0.49	2.43	5	1
1:A:173:MET:SD	1:A:178:LEU:HB2	0.47	2.49	3	3
1:A:261:LEU:HD23	1:A:334:ARG:HA	0.46	1.86	3	1
1:A:155:ASN:ND2	1:A:223:SER:HB3	0.46	2.25	10	2
1:A:251:SER:O	1:A:287:ALA:HA	0.46	2.11	3	2
1:A:214:PHE:O	1:A:217:ILE:HG22	0.46	2.10	5	4
1:A:273:ASP:HA	1:A:276:LYS:NZ	0.45	2.27	2	2
1:A:170:ASN:OD1	1:A:185:PRO:HD2	0.45	2.12	10	1
1:A:293:ASP:HB3	1:A:296:THR:OG1	0.45	2.12	4	3
1:A:263:ILE:HA	1:A:331:LEU:O	0.45	2.12	2	1
1:A:173:MET:HE1	1:A:211:ALA:HB2	0.45	1.89	10	1
1:A:263:ILE:O	1:A:302:TYR:HA	0.44	2.12	4	2
1:A:253:VAL:O	1:A:255:PRO:HD3	0.44	2.12	8	2
1:A:288:PHE:CD1	1:A:305:CYS:HB3	0.44	2.47	2	2
1:A:212:MET:HE2	1:A:226:ILE:HG22	0.44	1.88	10	1
1:A:254:VAL:HG12	1:A:260:LYS:HZ1	0.44	1.73	5	1
1:A:261:LEU:CD2	1:A:334:ARG:HA	0.44	2.42	3	1
1:A:309:ASP:HB2	1:A:312:VAL:HG12	0.43	1.91	8	1
1:A:257:SER:O	1:A:260:LYS:HB2	0.43	2.13	5	1
1:A:178:LEU:HB3	1:A:210:GLN:OE1	0.43	2.13	5	1
1:A:152:TYR:O	1:A:226:ILE:HA	0.43	2.14	2	3
1:A:309:ASP:HB3	1:A:312:VAL:HG12	0.42	1.91	9	1
1:A:290:LEU:HD22	1:A:303:ALA:HB2	0.42	1.90	9	1
1:A:288:PHE:CD2	1:A:305:CYS:HB3	0.42	2.49	6	2
1:A:206:ASP:O	1:A:209:THR:HG22	0.42	2.15	7	1
1:A:322:GLY:O	1:A:329:LYS:HD2	0.42	2.14	7	1
1:A:266:LEU:HB2	1:A:301:GLY:HA2	0.42	1.92	9	2
1:A:331:LEU:HD13	1:A:331:LEU:C	0.41	2.35	2	2
1:A:316:ALA:O	1:A:320:LEU:HG	0.41	2.15	9	1
1:A:263:ILE:HD11	1:A:305:CYS:SG	0.41	2.56	8	1
1:A:321:ASN:HB2	1:A:332:VAL:O	0.41	2.16	4	1
1:A:150:ARG:HG2	1:A:201:GLU:HA	0.40	1.92	3	1
1:A:309:ASP:O	1:A:312:VAL:HG12	0.40	2.16	4	1
1:A:331:LEU:C	1:A:331:LEU:HD13	0.40	2.37	7	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	165/205 (80%)	162±1 (98±1%)	3±1 (2±1%)	0±0 (0±0%)	54 85
All	All	1650/2050 (80%)	1619 (98%)	30 (2%)	1 (0%)	54 85

All 1 unique Ramachandran outliers are listed below.

Mol	Chain	Res	Type	Models (Total)
1	A	335	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	135/168 (80%)	128±2 (95±1%)	7±2 (5±1%)	26 75
All	All	1350/1680 (80%)	1277 (95%)	73 (5%)	26 75

All 25 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	179	THR	10
1	A	324	GLN	10
1	A	173	MET	6
1	A	325	LEU	6
1	A	146	ARG	4
1	A	306	GLU	4
1	A	189	VAL	3
1	A	200	LEU	3
1	A	286	LYS	3
1	A	334	ARG	3
1	A	224	LEU	3
1	A	168	PHE	3
1	A	190	GLN	2
1	A	276	LYS	2
1	A	274	GLN	1

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Mol	Chain	Res	Type	Models (Total)
1	A	311	ASN	1
1	A	333	GLN	1
1	A	167	ASP	1
1	A	165	MET	1
1	A	328	LYS	1
1	A	180	GLN	1
1	A	261	LEU	1
1	A	290	LEU	1
1	A	172	GLN	1
1	A	272	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 84% for the well-defined parts and 82% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *U2AF65_nxRRM12_assignments_new.txt*

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	2243
Number of shifts mapped to atoms	2243
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 (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	199	2.77 ± 0.11	Should be applied
$^{13}C_{\beta}$	181	2.85 ± 0.14	Should be applied
$^{13}C'$	0	—	None (insufficient data)
^{15}N	188	0.23 ± 0.26	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 84%, i.e. 1888 atoms were assigned a chemical shift out of a possible 2241. 0 out of 30 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	657/828 (79%)	336/339 (99%)	163/330 (49%)	158/159 (99%)
Sidechain	1109/1249 (89%)	757/816 (93%)	330/384 (86%)	22/49 (45%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	122/164 (74%)	69/80 (86%)	53/82 (65%)	0/2 (0%)
Overall	1888/2241 (84%)	1162/1235 (94%)	546/796 (69%)	180/210 (86%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 82%, i.e. 2243 atoms were assigned a chemical shift out of a possible 2742. 0 out of 36 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	792/1022 (77%)	405/418 (97%)	199/410 (49%)	188/194 (97%)
Sidechain	1309/1530 (86%)	888/999 (89%)	394/471 (84%)	27/60 (45%)
Aromatic	142/190 (75%)	79/92 (86%)	63/94 (67%)	0/4 (0%)
Overall	2243/2742 (82%)	1372/1509 (91%)	656/975 (67%)	215/258 (83%)

7.1.4 Statistically unusual chemical shifts (i)

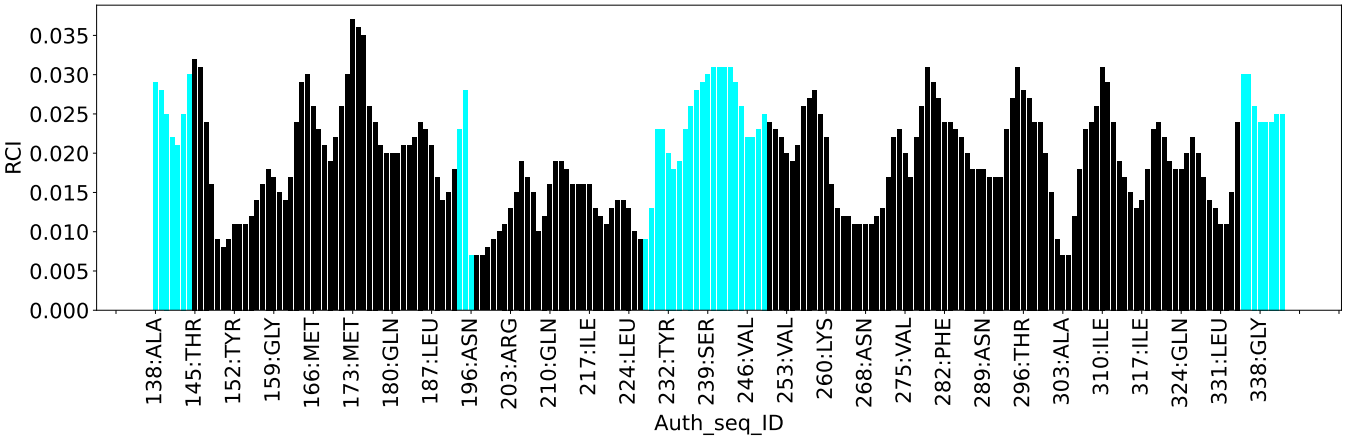
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	172	GLN	HG2	0.65	1.01 – 3.62	-6.4
1	A	208	THR	HA	2.02	2.11 – 6.79	-5.2

7.1.5 Random Coil Index (RCI) plots (i)

The image below reports *random coil index* values for the protein chains in the structure. The height of each bar gives a probability of a given residue to be disordered, as predicted from the available chemical shifts and the amino acid sequence. A value above 0.2 is an indication of significant predicted disorder. The colour of the bar shows whether the residue is in the well-defined core (black) or in the ill-defined residue ranges (cyan), as described in section 2 on ensemble composition.If well-defined core and ill-defined regions are not identified then it is shown as gray bars.

Random coil index (RCI) for chain A:


RCI
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
138:ALA
145:THR
152:TYR
159:GLY
166:MET
173:MET
180:GLN
187:LEU
196:ASN
203:ARG
210:GLN
217:ILE
224:LEU
232:TYR
239:SER
246:VAL
253:VAL
260:LYS
268:ASN
275:VAL
282:PHE
289:ASN
296:THR
303:ALA
310:ILE
317:ILE
324:GLN
331:LEU
338:GLY
Auth_seq_ID

8 NMR restraints analysis (i)

No restraints data found

9 Distance violation analysis (i)

No distance restraints data found

10 Dihedral-angle violation analysis (i)

Dihedral angle analysis failed due to data error in the dihedral angle restraints, possibly missing target value