wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	2MBZ
BMRB ID	:	19421
Title	:	Structural Basis of a Thiopeptide Antibiotic Multidrug Resistance System from Streptomyces lividans:Promothiocin A in Complex with TipAS
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Deposited on	:	2013-08-12

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

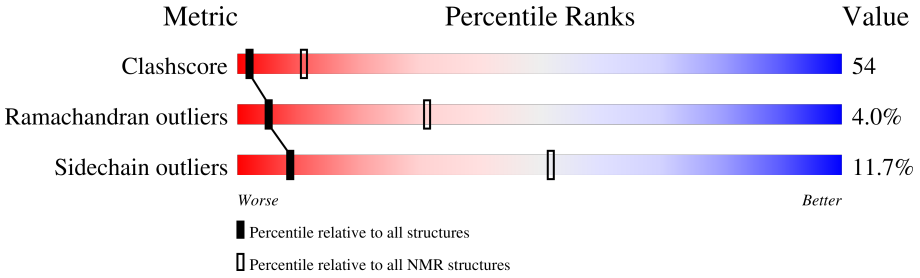
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36

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	144	28% 53% 6% 12% •
2	B	12	42% 8% 50%

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:113-A:126, A:142-A:253, B:501-B:501, B:503-B:504, B:506-B:506, B:508-B:508, B:511-B:511 (132)	0.62	2

Ill-defined regions of proteins are excluded from the global statistics.

Ligands and non-protein polymers are included in the analysis.

The models can be grouped into 1 clusters and 1 single-model cluster was found.

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

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 2302 atoms, of which 1091 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called HTH-type transcriptional activator TipA.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	143	2207	706	1053	204	237	7	0

- Molecule 2 is a protein called Promothiocin A.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
2	B	12	95	36	38	11	8	2	1

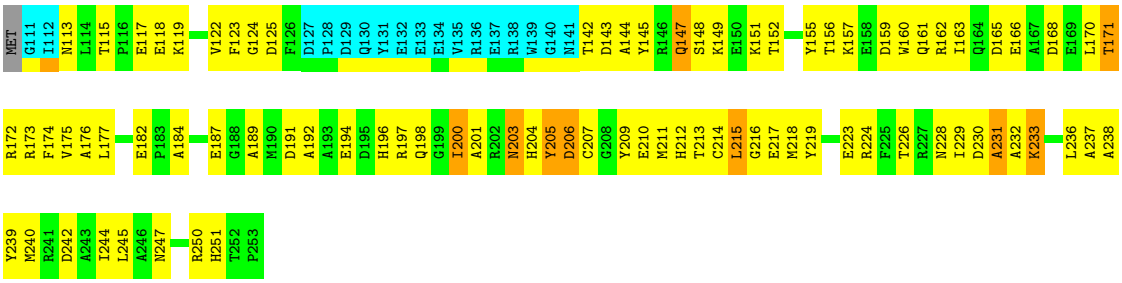
4 Residue-property plots (i)

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.

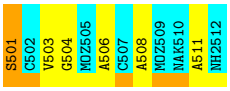
• Molecule 1: HTH-type transcriptional activator TipA

Chain A: 28% 53% 6% 12%



• Molecule 2: Promothiocin A

Chain B: 42% 8% 50%

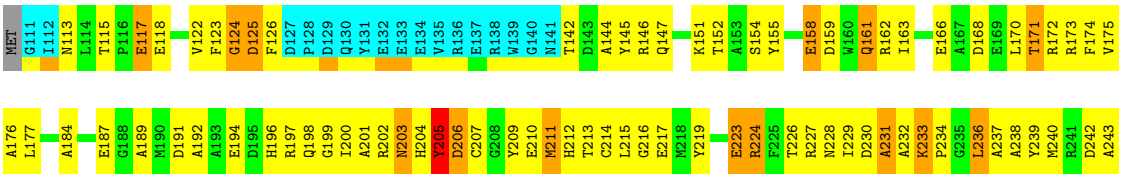


4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 2. Colouring as in section 4.1 above.

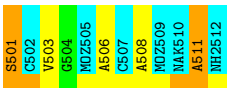
• Molecule 1: HTH-type transcriptional activator TipA

Chain A: 31% 47% 10% 12%



- Molecule 2: Promothiocin A

Chain B:



5 Refinement protocol and experimental data overview (i)

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

Of the 100 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
X-PLOR NIH	structure solution	2.30
X-PLOR NIH	refinement	2.30

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	1737
Number of shifts mapped to atoms	1733
Number of unparsed shifts	0
Number of shifts with mapping errors	4
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: NAK, MOZ, NH2, BB9

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	0.28±0.01	0±0/1027 (0.0± 0.0%)	0.41±0.01	0±0/1384 (0.0± 0.0%)
2	B	2.61±0.06	1±0/21 (4.8± 0.0%)	1.36±0.05	1±0/22 (4.5± 0.0%)
All	All	0.46	10/10480 (0.1%)	0.44	10/14060 (0.1%)

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
2	B	0.4±0.5	0.0±0.0
All	All	4	0

All unique bond outliers are listed below.

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)	Models	
								Worst	Total
2	B	501	SER	CA-CB	-11.03	1.36	1.52	10	10

All unique angle outliers are listed below.

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
2	B	501	SER	CB-CA-C	5.97	121.44	110.10	3	10

All unique chiral outliers are listed below.

Mol	Chain	Res	Type	Atoms	Models (Total)
2	B	501	SER	CA	4

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	1007	929	928	106±12
2	B	26	27	27	5±2
All	All	10330	9560	9550	1075

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 54.

5 of 514 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:204:HIS:O	1:A:205:TYR:CD2	1.07	2.06	7	1
1:A:215:LEU:HD12	1:A:216:GLY:N	0.96	1.74	9	5
1:A:236:LEU:HD23	1:A:237:ALA:H	0.95	1.21	6	8
1:A:204:HIS:O	1:A:205:TYR:CG	0.94	2.19	7	1
1:A:229:ILE:HG22	1:A:236:LEU:HD11	0.90	1.44	7	7

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	125/144 (87%)	114±2 (92±2%)	7±2 (5±1%)	4±1 (3±1%)	7	39
2	B	5/12 (42%)	1±1 (14±20%)	3±1 (60±18%)	1±0 (26±9%)	0	1
All	All	1300/1560 (83%)	1151 (89%)	97 (7%)	52 (4%)	5	31

5 of 11 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	231	ALA	10
1	A	206	ASP	9
2	B	506	ALA	8
1	A	205	TYR	7
1	A	125	ASP	6

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	102/118 (86%)	90±3 (88±2%)	12±3 (12±2%)	8 51
2	B	1/2 (50%)	1±0 (100±0%)	0±0 (0±0%)	100 100
All	All	1030/1200 (86%)	910 (88%)	120 (12%)	9 52

5 of 45 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	233	LYS	9
1	A	194	GLU	9
1	A	200	ILE	8
1	A	171	THR	8
1	A	203	ASN	7

6.3.3 RNA (i)

There are no RNA molecules in this entry.

6.4 Non-standard residues in protein, DNA, RNA chains (i)

4 non-standard protein/DNA/RNA residues 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	BB9	B	507	2	3,5,6	1.78±0.06	1±0 (33±0%)
2	MOZ	B	509	2	4,5,7	2.35±0.03	2±0 (50±0%)
2	BB9	B	502	2	3,5,6	1.49±0.07	1±0 (33±0%)
2	MOZ	B	505	2	6,6,7	2.66±0.10	3±0 (46±6%)

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	BB9	B	507	2	1,5,7	3.69±0.27	1±0 (100±0%)
2	MOZ	B	509	2	3,6,9	3.76±0.13	2±0 (66±0%)
2	BB9	B	502	2	1,5,7	3.07±0.14	1±0 (100±0%)
2	MOZ	B	505	2	4,7,9	8.84±0.04	4±0 (100±0%)

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	BB9	B	507	2	-	0±0,0,4,6	-
2	BB9	B	502	2	-	0±0,0,4,6	-
2	MOZ	B	505	2	-	0±0,0,6,8	-
2	MOZ	B	509	2	-	0±0,0,4,8	-

5 of 7 unique bond 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(Å)	Ideal(Å)	Models	
								Worst	Total
2	B	505	MOZ	C-CA	4.89	1.53	1.45	1	10
2	B	509	MOZ	OG-CB	4.05	1.42	1.31	10	10

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Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)	Models	
								Worst	Total
2	B	505	MOZ	OG-CB	3.69	1.41	1.31	2	10
2	B	507	BB9	CA-N	3.11	1.43	1.35	1	10
2	B	509	MOZ	CA-N	2.79	1.40	1.33	8	10

5 of 8 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
2	B	505	MOZ	OG-CB-CA	16.29	106.29	121.10	3	10
2	B	505	MOZ	C6-CB-CA	5.77	132.65	124.02	1	10
2	B	509	MOZ	OG-CB-C6	4.74	124.21	114.59	1	10
2	B	509	MOZ	C-CA-N	4.71	120.43	115.40	4	10
2	B	507	BB9	O-C-CA	4.02	120.28	125.39	9	10

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

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 88% 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	1737
Number of shifts mapped to atoms	1733
Number of unparsed shifts	0
Number of shifts with mapping errors	4
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	8

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

- No matching atom found in the structure. All 4 occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	B	501	SER	HB2	7.77	0.006	1
1	B	511	DHA	H	5.943	0.008	1
1	B	511	DHA	HB1	2.837	0.001	1
1	B	511	DHA	HB2	2.553	0.004	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
$^{13}C_{\alpha}$	143	-0.64 ± 0.18	Should be checked
$^{13}C_{\beta}$	134	0.21 ± 0.05	None needed (< 0.5 ppm)
$^{13}C'$	133	-0.55 ± 0.12	Should be applied
^{15}N	137	0.28 ± 0.33	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. 1537 atoms were assigned a chemical shift out of a possible 1724. 0 out of 11 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	632/660 (96%)	264/268 (99%)	246/264 (93%)	122/128 (95%)
Sidechain	778/919 (85%)	532/590 (90%)	230/286 (80%)	16/43 (37%)
Aromatic	127/145 (88%)	65/71 (92%)	60/68 (88%)	2/6 (33%)
Overall	1537/1724 (89%)	861/929 (93%)	536/618 (87%)	140/177 (79%)

7.1.4 Statistically unusual chemical shifts (i)

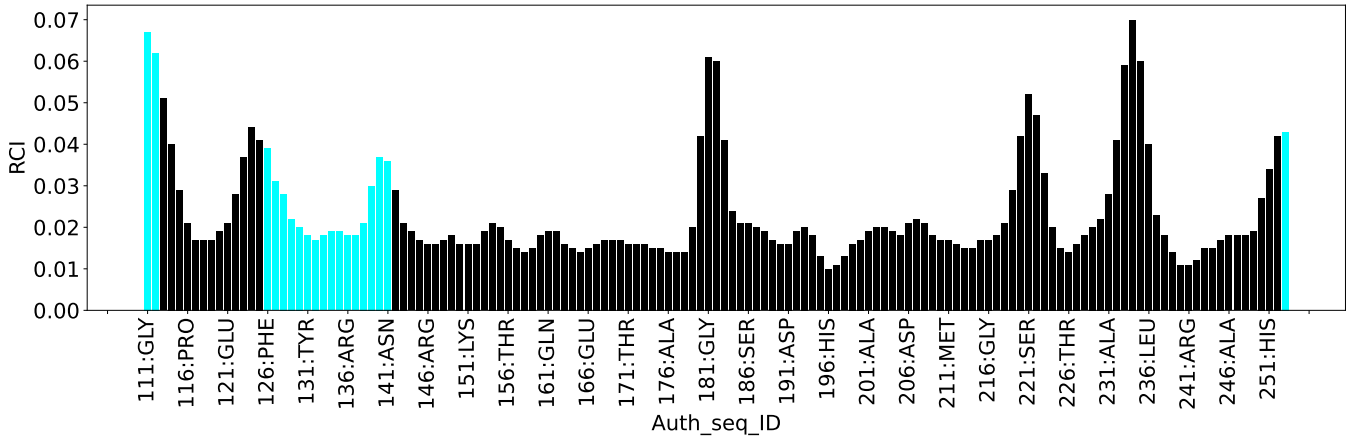
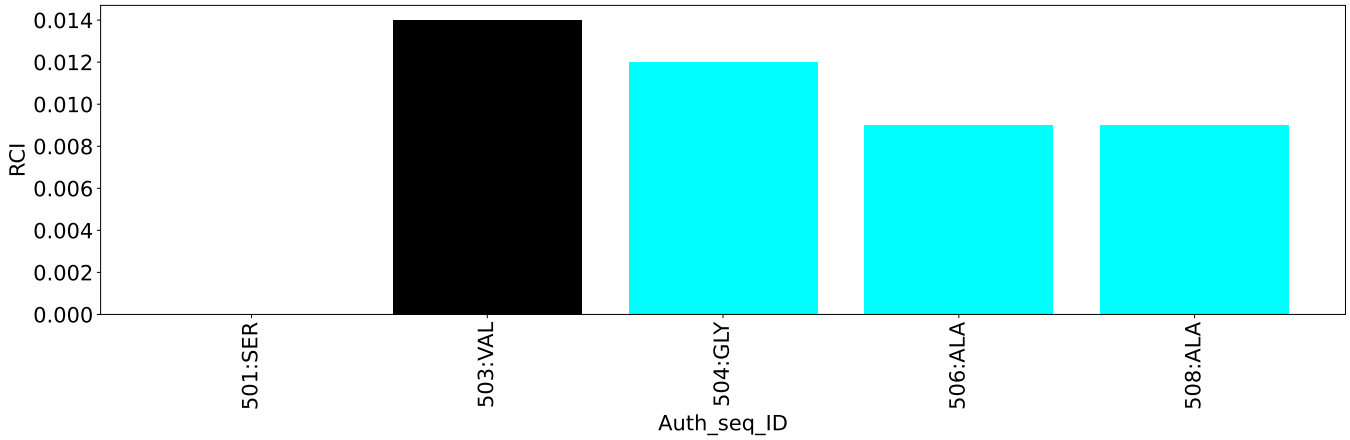
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	B	501	SER	HB2	7.77	2.61 – 5.13	15.5
1	B	501	SER	HB3	7.77	2.49 – 5.20	14.5
1	A	138	ARG	HB2	-0.06	0.52 – 3.08	-7.2
1	A	138	ARG	HB3	0.02	0.43 – 3.11	-6.5
1	B	503	VAL	HG11	2.42	-0.48 – 2.12	6.1
1	B	503	VAL	HG12	2.42	-0.48 – 2.12	6.1
1	B	503	VAL	HG13	2.42	-0.48 – 2.12	6.1
1	B	504	GLY	HA2	1.79	2.15 – 5.77	-6.0

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 A:



Random coil index (RCI) for chain B: