



# wwPDB NMR Structure Validation Summary Report ⓘ

Jul 8, 2019 – 02:31 PM EDT

PDB ID : 6FE6  
Title : Solution structure of a last generation P2-P4 macrocyclic inhibitor  
Authors : Gallo, M.; Eliseo, T.; Cicero, D.O.  
Deposited on : 2017-12-29

This is a wwPDB NMR Structure Validation Summary Report for a publicly released PDB entry.

We welcome your comments at [validation@mail.wwpdb.org](mailto: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 ⓘ symbol.

---

The following versions of software and data (see [references ⓘ](#)) were used in the production of this report:

Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	1.8.0 (224370), CSD as540be (2019)
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20171227.v01 (using entries in the PDB archive December 27th 2017)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	2.3.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.3.2

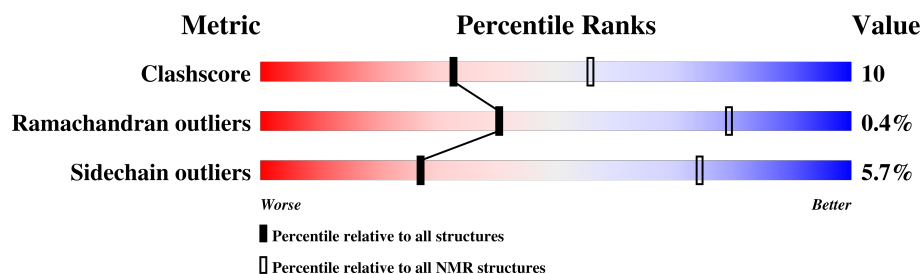
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*SOLUTION NMR*

The overall completeness of chemical shifts assignment is 75%.

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	136327	12091
Ramachandran outliers	132723	10835
Sidechain outliers	132532	10811

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  $\geq 3$ , 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  $\leq 5\%$ .

Mol	Chain	Length	Quality of chain
1	A	165	 70% 14% 13% .

The following table lists non-polymeric compounds, carbohydrate monomers and non-standard residues in protein, DNA and RNA chains that are outliers for geometric criteria:

Mol	Chain	Compound	Res	Total models with violations	
				Chirality	Geometry
2	A	4P2	201	18	-

## 2 Ensemble composition and analysis

This entry contains 18 models. Model 7 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:32-A:60, A:72-A:181 (139)	0.47	7

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 4 clusters and 1 single-model cluster was found.

Cluster number	Models
1	10, 11, 13, 14, 15, 16
2	3, 5, 12, 17, 18
3	1, 6, 7
4	4, 8, 9
Single-model clusters	2

### 3 Entry composition [i](#)

There are 3 unique types of molecules in this entry. The entry contains 2458 atoms, of which 1226 are hydrogens and 0 are deuteriums.

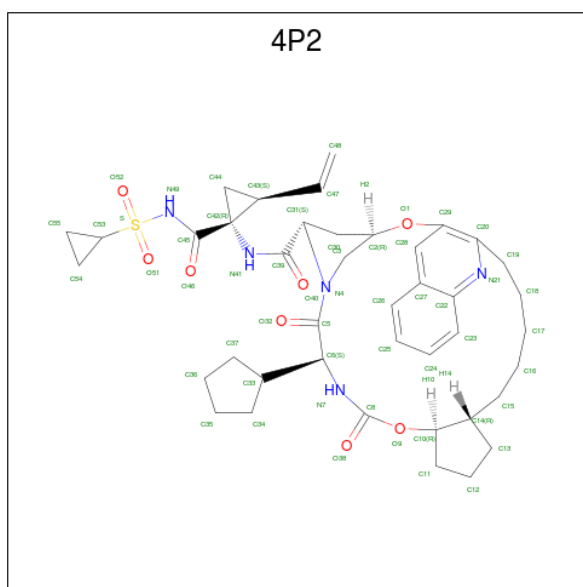
- Molecule 1 is a protein called Non-structural 3 protease.

Mol	Chain	Residues	Atoms						Trace
1	A	160	Total	C	H	N	O	S	0
			2350	729	1174	215	223	9	

There are 6 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	181	ALA	SER	conflict	UNP A6N4I5
A	182	SER	-	expression tag	UNP A6N4I5
A	183	LYS	-	expression tag	UNP A6N4I5
A	184	LYS	-	expression tag	UNP A6N4I5
A	185	LYS	-	expression tag	UNP A6N4I5
A	186	LYS	-	expression tag	UNP A6N4I5

- Molecule 2 is (3aR,7S,10S,12R,24aR)-7-cyclopentyl-N-{(1R,2S)-1-[(cyclopropylsulfonyl)carbonyl]-2-ethenylcyclopropyl}-5,8-dioxo-1,2,3,3a,5,6,7,8,11,12,20,21,22,23,24,24a-hexadecahydro-10H-9,12-methanocyclopenta[18,19][1,10,3,6]dioxadiazacyclononadecino[12,11-b]quinolin-10-carboxamide (three-letter code: 4P2) (formula: C<sub>41</sub>H<sub>53</sub>N<sub>5</sub>O<sub>8</sub>S).



Mol	Chain	Residues	Atoms					
2	A	1	Total	C	H	N	O	S
			107	41	52	5	8	1

- Molecule 3 is ZINC ION (three-letter code: ZN) (formula: Zn).

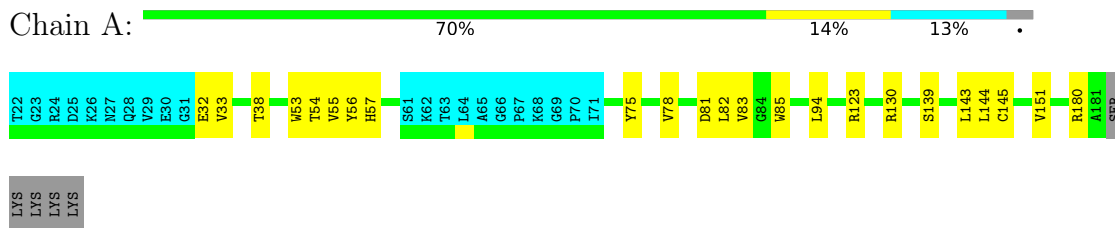
Mol	Chain	Residues	Atoms	
			Total	Zn
3	A	1	1	1

## 4 Residue-property plots [i](#)

### 4.1 Average score per residue in the NMR ensemble

These plots are provided for all protein, RNA and DNA chains in the entry. The first graphic is the same as shown in the summary in section 1 of this report. The second graphic shows the sequence where residues are colour-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. Stretches of 2 or more consecutive residues without any outliers are shown as green connectors. Residues which are classified as ill-defined in the NMR ensemble, are shown in cyan with an underline colour-coded according to the previous scheme. Residues which were present in the experimental sample, but not modelled in the final structure are shown in grey.

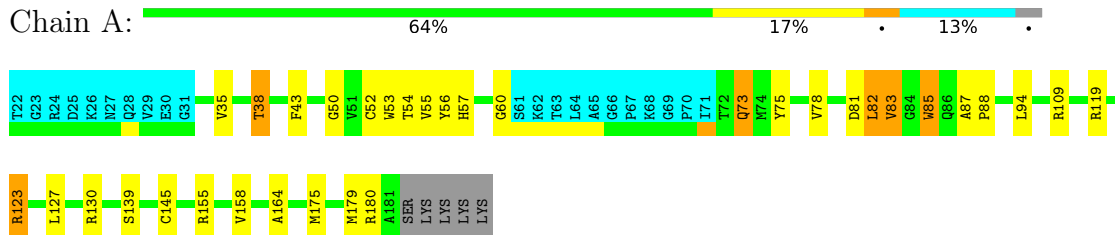
- Molecule 1: Non-structural 3 protease



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

The representative model is number 7. Colouring as in section 4.1 above.

- Molecule 1: Non-structural 3 protease



## 5 Refinement protocol and experimental data overview

The models were refined using the following method: *molecular dynamics*.

Of the 18 calculated structures, 18 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
Xplor-NIH	structure calculation	

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)	6fe6_cs.cif
Number of chemical shift lists	1
Total number of shifts	1574
Number of shifts mapped to atoms	1574
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	75%

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

## 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: ZN, 4P2

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	1.02±0.00	1±0/1048 (0.1±0.0%)	1.22±0.00	0±0/1430 (0.0±0.0%)
All	All	1.02	18/18864 (0.1%)	1.22	0/25740 (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	1.0±0.0
All	All	0	18

All unique bond outliers are listed below.

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)	Models	
								Worst	Total
1	A	81	ASP	CG-OD2	5.76	1.38	1.25	18	18

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	180	ARG	Sidechain	18

### 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	1026	1017	1018	21±9
2	A	55	52	0	1±0
3	A	1	0	0	0±1
All	All	19476	19242	18321	380

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 10.

5 of 203 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:145:CYS:SG	3:A:202:ZN:ZN	0.93	1.57	5	6
1:A:82:LEU:HD21	1:A:178:THR:HG21	0.86	1.45	8	1
1:A:54:THR:HG21	1:A:85:TRP:CH2	0.84	2.07	6	7
1:A:55:VAL:HG11	1:A:57:HIS:CE1	0.81	2.10	8	18
1:A:56:TYR:CE2	1:A:78:VAL:HG22	0.81	2.11	4	18

## 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	138/165 (84%)	130±2 (94±1%)	7±2 (5±1%)	1±1 (0±1%)	40	79
All	All	2484/2970 (84%)	2345 (94%)	130 (5%)	9 (0%)	40	79

All 5 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	113	VAL	3
1	A	100	GLY	2
1	A	32	GLU	2
1	A	60	GLY	1
1	A	114	ILE	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	113/134 (84%)	107±3 (94±2%)	6±3 (6±2%)	27 74
All	All	2034/2412 (84%)	1919 (94%)	115 (6%)	27 74

5 of 44 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	38	THR	13
1	A	145	CYS	8
1	A	123	ARG	8
1	A	119	ARG	8
1	A	82	LEU	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](#)

There are no non-standard protein/DNA/RNA residues in this entry.

## 6.5 Carbohydrates [i](#)

There are no carbohydrates in this entry.

## 6.6 Ligand geometry [i](#)

Of 2 ligands modelled in this entry, 1 is monoatomic - leaving 1 for Mogul analysis.

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	4P2	A	201	-	57,62,62	2.09±0.04	3±0 (5±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	4P2	A	201	-	67,92,92	1.00±0.03	0±0 (0±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	4P2	A	201	-	1±0,1,18,19	0±0,57,99,99	0±0,7,8,8

All 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	A	201	4P2	C20-N21	7.64	1.42	1.32	14	18
2	A	201	4P2	C28-C29	6.51	1.48	1.36	4	18
2	A	201	4P2	C27-C22	5.41	1.50	1.42	13	18

There are no bond-angle outliers.

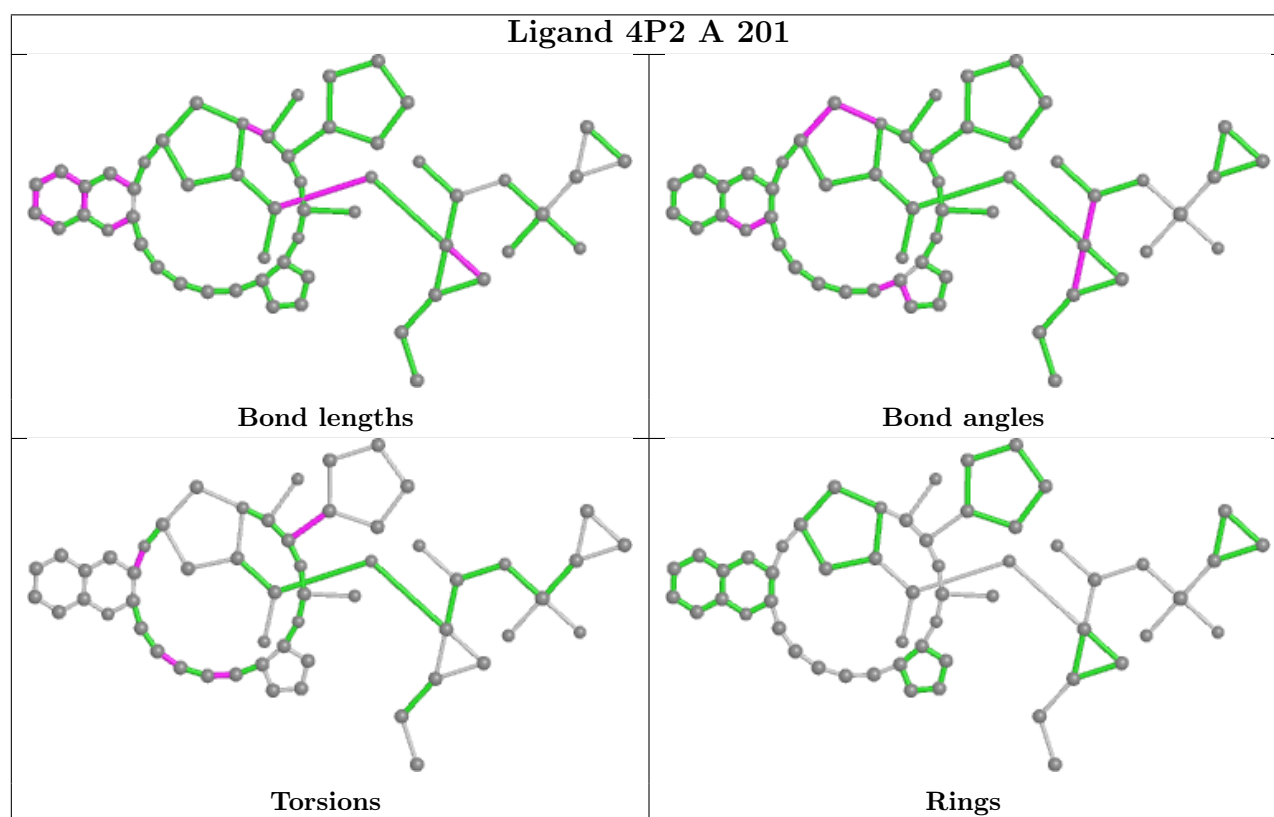
All unique chiral outliers are listed below.

Mol	Chain	Res	Type	Atoms	Models (Total)
2	A	201	4P2	N4	18

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 all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less than 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.



## 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

The completeness of assignment taking into account all chemical shift lists is 75% for the well-defined parts and 72% for the entire structure.

### 7.1 Chemical shift list 1

File name: 6fe6\_cs.cif

Chemical shift list name: *assigned\_chem\_shift\_list\_0*

#### 7.1.1 Bookkeeping

The following table shows the results of parsing the chemical shift list and reports the number of nuclei with statistically unusual chemical shifts.

Total number of shifts	1574
Number of shifts mapped to atoms	1574
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	6

#### 7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction $\pm$ precision, ppm	Suggested action
$^{13}\text{C}_\alpha$	148	$-0.58 \pm 0.09$	Should be applied
$^{13}\text{C}_\beta$	108	$-0.20 \pm 0.18$	None needed ( $< 0.5$ ppm)
$^{13}\text{C}'$	127	$-0.09 \pm 0.19$	None needed ( $< 0.5$ ppm)
$^{15}\text{N}$	129	$0.59 \pm 0.45$	None needed (imprecise)

#### 7.1.3 Completeness of resonance assignments

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 75%, i.e. 1186 atoms were assigned a chemical shift out of a possible 1585. 25 out of 28 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^1\text{H}$	$^{13}\text{C}$	$^{15}\text{N}$
Backbone	582/677 (86%)	242/269 (90%)	232/278 (83%)	108/130 (83%)
Sidechain	536/801 (67%)	344/469 (73%)	192/293 (66%)	0/39 (0%)

*Continued on next page...*

Continued from previous page...

	Total	<sup>1</sup> H	<sup>13</sup> C	<sup>15</sup> N
Aromatic	68/107 (64%)	41/55 (75%)	19/44 (43%)	8/8 (100%)
Overall	1186/1585 (75%)	627/793 (79%)	443/615 (72%)	116/177 (66%)

### 7.1.4 Statistically unusual chemical shifts [i](#)

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

Mol	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	A	68	LYS	CD	42.40	34.86 – 23.06	11.4
1	A	68	LYS	HD3	3.08	2.75 – 0.45	6.4
1	A	68	LYS	HD2	3.08	2.76 – 0.46	6.4
1	A	58	GLY	HA3	1.78	5.80 – 2.00	-5.6
1	A	85	TRP	CZ2	122.60	121.76 – 106.66	5.6
1	A	85	TRP	CH2	113.90	133.06 – 114.56	-5.4

### 7.1.5 Random Coil Index (RCI) plots [i](#)

The image below reports *random coil index* values for the protein chains in the structure. The height of each bar gives a probability of a given residue to be disordered, as predicted from the available chemical shifts and the amino acid sequence. A value above 0.2 is an indication of significant predicted disorder. The colour of the bar shows whether the residue is in the well-defined core (black) or in the ill-defined residue ranges (cyan), as described in section 2 on ensemble composition.

Random coil index (RCI) for chain A:

