Full wwPDB NMR Structure Validation Report (i)

Jun 5, 2020 – 11:03 pm BST

PDB ID	:	6BHN
Title	:	Red Light-Absorbing State of NpR6012g4, a Red/Green Cyanobacteriochrome
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Deposited on	:	2017-10-31

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 following versions of software and data (see references (1)) were used in the production of this report:

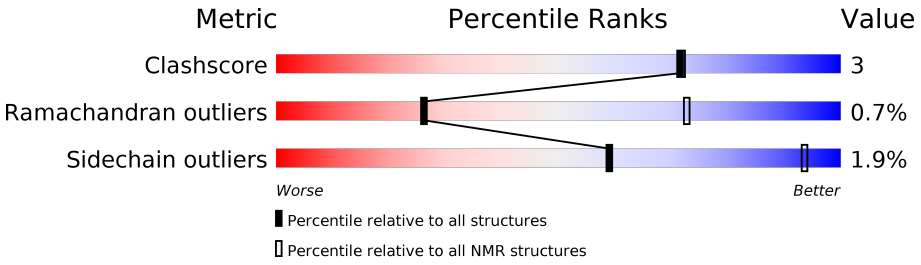
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	2.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.11

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	180	76% · 8% 13%

2 Ensemble composition and analysis

This entry contains 10 models. Model 5 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:600-A:643, A:653-A:749 (141)	0.45	5

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 3 clusters and 4 single-model clusters were found.

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

3 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 2552 atoms, of which 1248 are hydrogens and 0 are deuteriums.

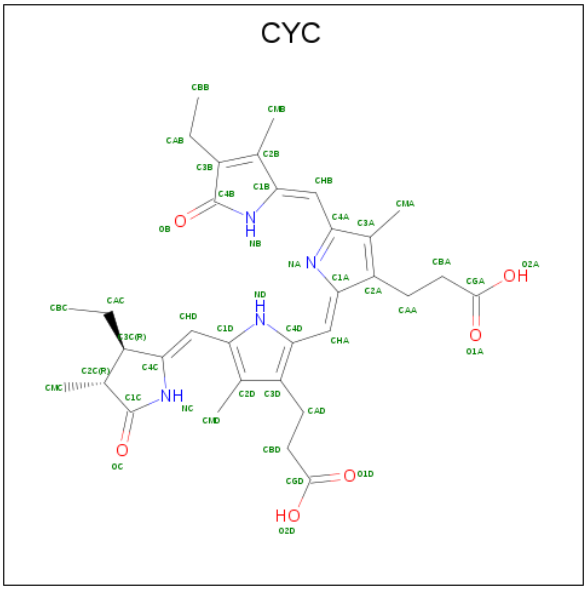
- Molecule 1 is a protein called Methyl-accepting chemotaxis sensory transducer with phytochrome sensor.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	156	2471	803	1210	214	242	2	0

There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	583	MET	-	initiating methionine	UNP B2IU14
A	584	GLY	-	expression tag	UNP B2IU14
A	761	PRO	-	expression tag	UNP B2IU14
A	762	GLY	-	expression tag	UNP B2IU14

- Molecule 2 is PHYCOCYANOBILIN (three-letter code: CYC) (formula: $C_{33}H_{40}N_4O_6$).



Mol	Chain	Residues	Atoms				
			Total	C	H	N	O
2	A	1	81	33	38	4	6

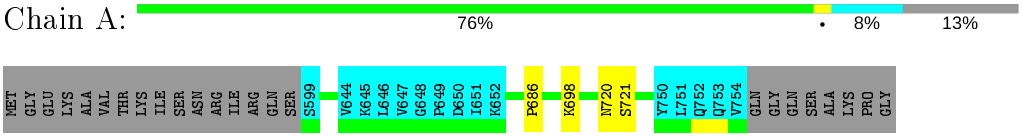
4 Residue-property plots (i)

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: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

Chain A:



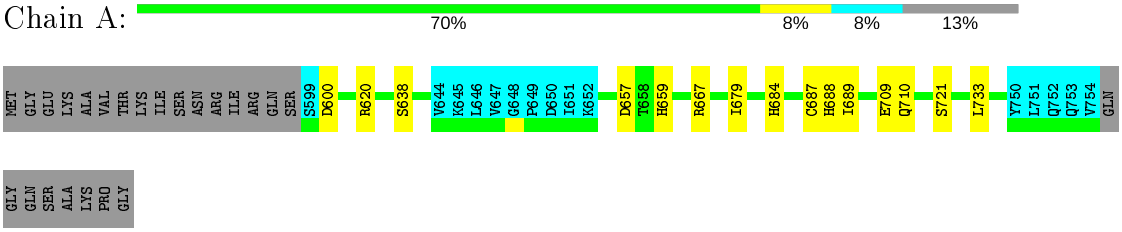
4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

• Molecule 1: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

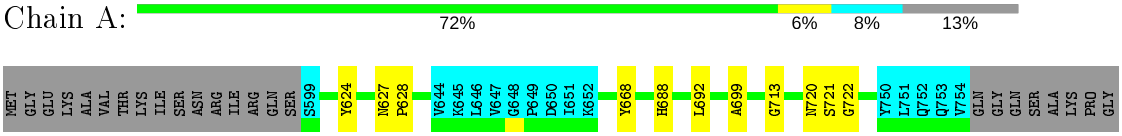
Chain A:



4.2.2 Score per residue for model 2

• Molecule 1: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

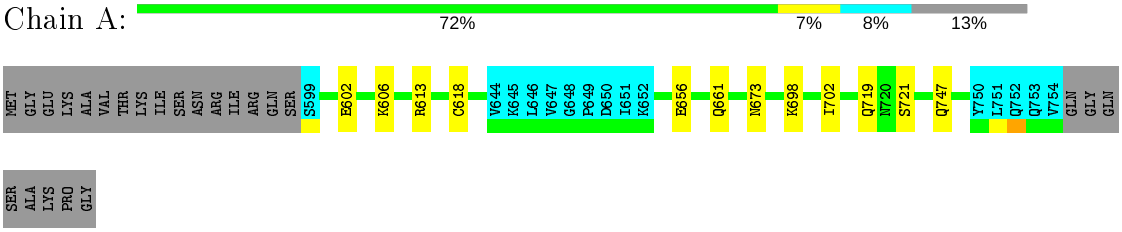
Chain A:



4.2.3 Score per residue for model 3

• Molecule 1: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

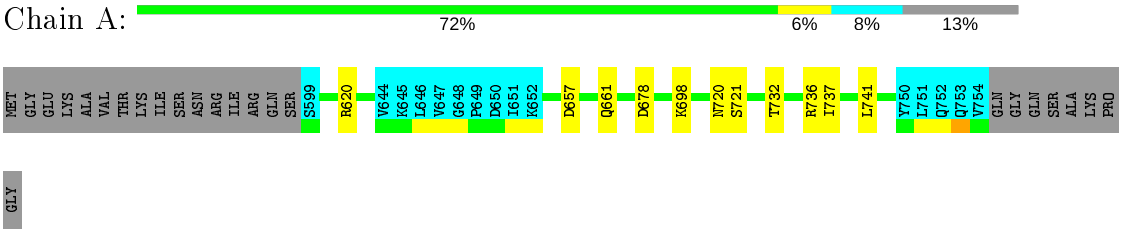
Chain A:



4.2.4 Score per residue for model 4

• Molecule 1: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

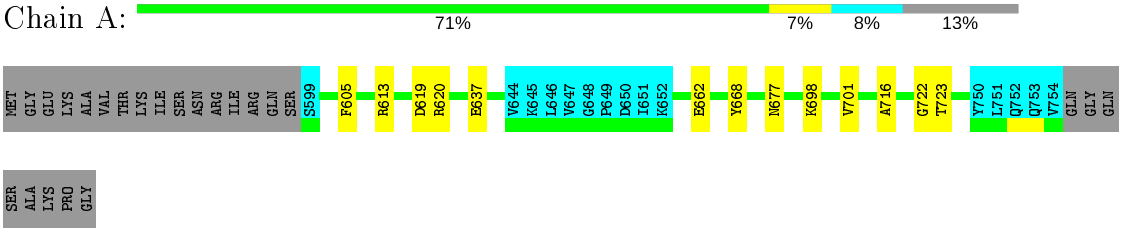
Chain A:



4.2.5 Score per residue for model 5 (medoid)

• Molecule 1: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

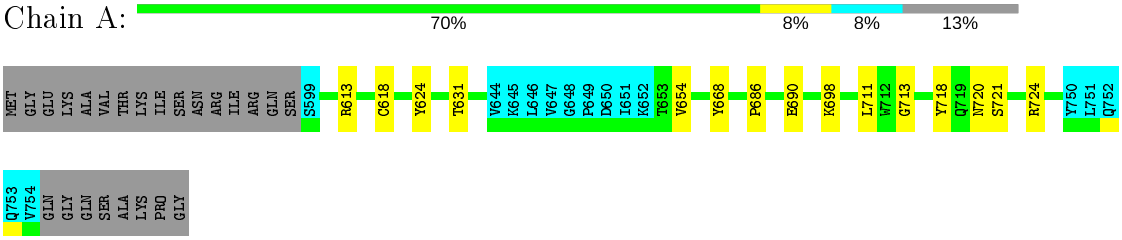
Chain A:



4.2.6 Score per residue for model 6

• Molecule 1: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

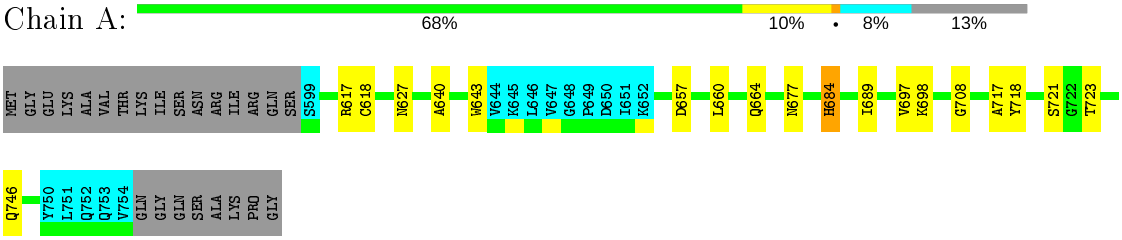
Chain A:



4.2.7 Score per residue for model 7

• Molecule 1: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

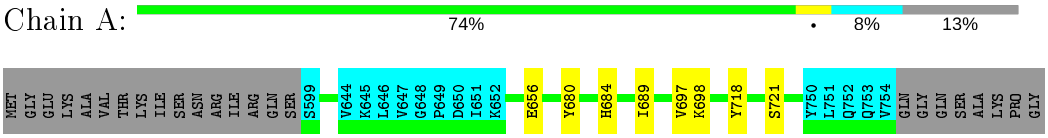
Chain A:



4.2.8 Score per residue for model 8

• Molecule 1: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

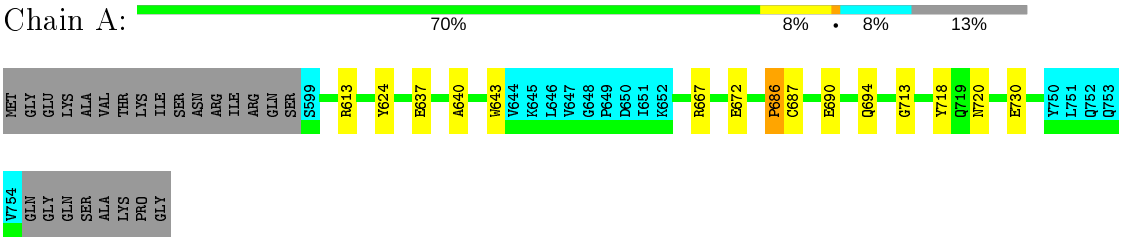
Chain A:



4.2.9 Score per residue for model 9

• Molecule 1: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

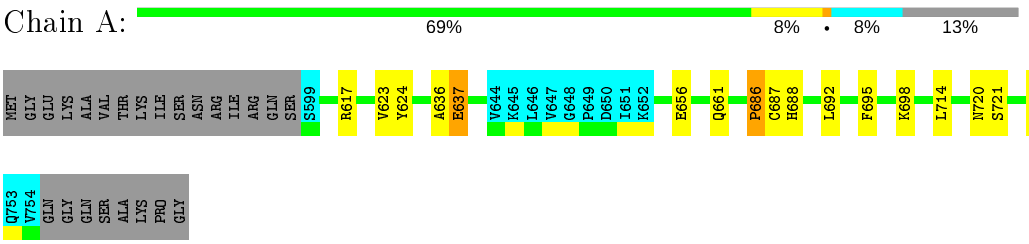
Chain A:



4.2.10 Score per residue for model 10

• Molecule 1: Methyl-accepting chemotaxis sensory transducer with phytochrome sensor

Chain A:



5 Refinement protocol and experimental data overview

The models were refined using the following method: *DGSA-distance geometry simulated annealing.*

Of the 200 calculated structures, 10 were deposited, based on the following criterion: *structures with the lowest energy.*

The following table shows the software used for structure solution, optimisation and refinement.

Software name	Classification	Version
X-PLOR NIH	refinement	2.44
NMRPipe	structure solution	8.9
Sparky	structure solution	3.12

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)	input_cs.cif
Number of chemical shift lists	1
Total number of shifts	2104
Number of shifts mapped to atoms	2104
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%

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

6 Model quality

6.1 Standard geometry

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

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

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	1143	1080	1080	6±1
2	A	43	38	37	2±2
All	All	11860	11180	11170	80

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 3.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
2:A:800:CYC:HBB3	2:A:800:CYC:HMB1	0.67	1.65	9	2
1:A:684:HIS:ND1	1:A:689:ILE:HB	0.64	2.07	8	1
2:A:800:CYC:CHD	2:A:800:CYC:HBC3	0.61	2.26	5	2
2:A:800:CYC:CBB	2:A:800:CYC:HMB1	0.61	2.26	2	2
2:A:800:CYC:HBC3	2:A:800:CYC:CHD	0.59	2.27	9	4
1:A:613:ARG:HA	1:A:618:CYS:SG	0.58	2.38	3	2
1:A:677:ASN:O	1:A:723:THR:HA	0.56	2.00	5	2
1:A:668:TYR:CE1	2:A:800:CYC:HMA3	0.55	2.37	6	2
1:A:620:ARG:HD2	1:A:637:GLU:OE1	0.54	2.03	5	1
1:A:657:ASP:O	1:A:661:GLN:HB2	0.54	2.02	4	1
1:A:688:HIS:O	1:A:692:LEU:HD23	0.53	2.03	2	2

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:732:THR:O	1:A:736:ARG:HG3	0.52	2.05	4	1
1:A:656:GLU:HG3	1:A:661:GLN:NE2	0.52	2.20	3	2
1:A:679:ILE:HB	1:A:689:ILE:HD12	0.51	1.82	1	1
1:A:698:LYS:N	1:A:721:SER:HA	0.51	2.20	8	4
1:A:709:GLU:HG3	1:A:710:GLN:OE1	0.50	2.07	1	1
2:A:800:CYC:HBC3	2:A:800:CYC:HHD	0.49	1.83	5	5
1:A:678:ASP:OD1	1:A:698:LYS:HE2	0.49	2.08	4	1
1:A:657:ASP:OD1	1:A:659:HIS:HB3	0.49	2.08	1	1
1:A:624:TYR:CZ	1:A:714:LEU:HB2	0.48	2.43	10	1
1:A:697:VAL:HG21	1:A:718:TYR:HB3	0.48	1.86	8	2
2:A:800:CYC:HMA1	2:A:800:CYC:NB	0.47	2.24	4	1
1:A:698:LYS:HB2	1:A:722:GLY:O	0.47	2.10	5	1
1:A:618:CYS:SG	1:A:717:ALA:HB1	0.47	2.50	7	1
1:A:697:VAL:HA	1:A:721:SER:H	0.46	1.71	8	1
1:A:620:ARG:HA	1:A:638:SER:O	0.46	2.10	1	1
1:A:624:TYR:O	1:A:713:GLY:HA3	0.45	2.11	2	3
1:A:667:ARG:HD3	1:A:672:GLU:OE1	0.45	2.11	9	1
1:A:708:GLY:HA2	1:A:746:GLN:OE1	0.45	2.12	7	1
2:A:800:CYC:CHD	2:A:800:CYC:CBC	0.45	2.95	10	2
1:A:684:HIS:CE1	1:A:689:ILE:HG12	0.45	2.46	7	1
1:A:680:TYR:HA	1:A:684:HIS:CD2	0.44	2.47	8	1
1:A:613:ARG:NH2	1:A:640:ALA:HB2	0.44	2.27	9	1
1:A:640:ALA:HB3	1:A:643:TRP:CD1	0.44	2.47	9	2
1:A:668:TYR:HE1	2:A:800:CYC:HMA3	0.44	1.73	2	1
2:A:800:CYC:CBC	2:A:800:CYC:CHD	0.44	2.96	4	2
1:A:673:ASN:HB2	1:A:702:ILE:O	0.44	2.11	3	1
1:A:623:VAL:HB	1:A:636:ALA:HB3	0.44	1.89	10	1
1:A:698:LYS:H	1:A:721:SER:HA	0.43	1.73	10	3
1:A:618:CYS:HA	1:A:719:GLN:HB3	0.43	1.90	3	1
1:A:701:VAL:O	1:A:716:ALA:HA	0.43	2.13	5	1
1:A:699:ALA:HB3	1:A:722:GLY:O	0.43	2.13	2	1
1:A:657:ASP:OD1	1:A:660:LEU:HG	0.43	2.13	7	1
1:A:687:CYS:O	1:A:688:HIS:HB3	0.43	2.13	1	1
1:A:686:PRO:O	1:A:687:CYS:HB3	0.42	2.15	9	2
1:A:613:ARG:HD2	1:A:619:ASP:O	0.42	2.14	5	1
1:A:737:ILE:O	1:A:741:LEU:HG	0.42	2.14	4	1
1:A:627:ASN:HB2	1:A:628:PRO:HD2	0.42	1.90	2	1
1:A:690:GLU:O	1:A:694:GLN:HG3	0.42	2.15	9	1
1:A:668:TYR:HE1	2:A:800:CYC:HAA2	0.42	1.75	5	1
2:A:800:CYC:HHD	2:A:800:CYC:HBC3	0.41	1.91	2	1
1:A:686:PRO:O	1:A:690:GLU:HG2	0.40	2.16	6	1

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:631:THR:O	1:A:654:VAL:HG22	0.40	2.17	6	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	141/180 (78%)	135±1 (96±1%)	5±1 (3±1%)	1±1 (1±1%)	26	73
All	All	1410/1800 (78%)	1352 (96%)	48 (3%)	10 (1%)	26	73

All 3 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	720	ASN	5
1	A	721	SER	3
1	A	686	PRO	2

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	121/154 (79%)	119±2 (98±1%)	2±2 (2±1%)	59	93
All	All	1210/1540 (79%)	1187 (98%)	23 (2%)	59	93

All 19 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	684	HIS	2

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Mol	Chain	Res	Type	Models (Total)
1	A	637	GLU	2
1	A	617	ARG	2
1	A	718	TYR	2
1	A	605	PHE	1
1	A	711	LEU	1
1	A	627	ASN	1
1	A	606	LYS	1
1	A	733	LEU	1
1	A	727	ASP	1
1	A	656	GLU	1
1	A	667	ARG	1
1	A	730	GLU	1
1	A	600	ASP	1
1	A	724	ARG	1
1	A	695	PHE	1
1	A	662	GLU	1
1	A	698	LYS	1
1	A	664	GLN	1

6.3.3 RNA

There are no RNA molecules in this entry.

6.4 Non-standard residues in protein, DNA, RNA chains

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

6.5 Carbohydrates

There are no carbohydrates in this entry.

LIGAND-GEOMETRY INFOmissingINFO

6.6 Other polymers

There are no such molecules in this entry.

6.7 Polymer linkage issues

There are no chain breaks in this entry.

7 Chemical shift validation

The completeness of assignment taking into account all chemical shift lists is 84% for the well-defined parts and 85% for the entire structure.

7.1 Chemical shift list 1

File name: input_cs.cif

Chemical shift list name: *assigned_chem_shift_list_1*

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	2104
Number of shifts mapped to atoms	2104
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	10

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	175	-0.37 ± 0.16	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	154	0.18 ± 0.17	None needed (< 0.5 ppm)
$^{13}C'$	173	-0.02 ± 0.22	None needed (< 0.5 ppm)
^{15}N	167	0.22 ± 0.31	None needed (< 0.5 ppm)

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 84%, i.e. 1470 atoms were assigned a chemical shift out of a possible 1745. 27 out of 27 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	690/699 (99%)	273/279 (98%)	280/282 (99%)	137/138 (99%)
Sidechain	677/864 (78%)	399/498 (80%)	259/325 (80%)	19/41 (46%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	103/182 (57%)	70/96 (73%)	27/77 (35%)	6/9 (67%)
Overall	1470/1745 (84%)	742/873 (85%)	566/684 (83%)	162/188 (86%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 85%, i.e. 1645 atoms were assigned a chemical shift out of a possible 1936. 32 out of 32 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	762/772 (99%)	301/308 (98%)	310/312 (99%)	151/152 (99%)
Sidechain	774/974 (79%)	459/562 (82%)	296/367 (81%)	19/45 (42%)
Aromatic	109/190 (57%)	74/100 (74%)	29/81 (36%)	6/9 (67%)
Overall	1645/1936 (85%)	834/970 (86%)	635/760 (84%)	176/206 (85%)

7.1.4 Statistically unusual chemical shifts (i)

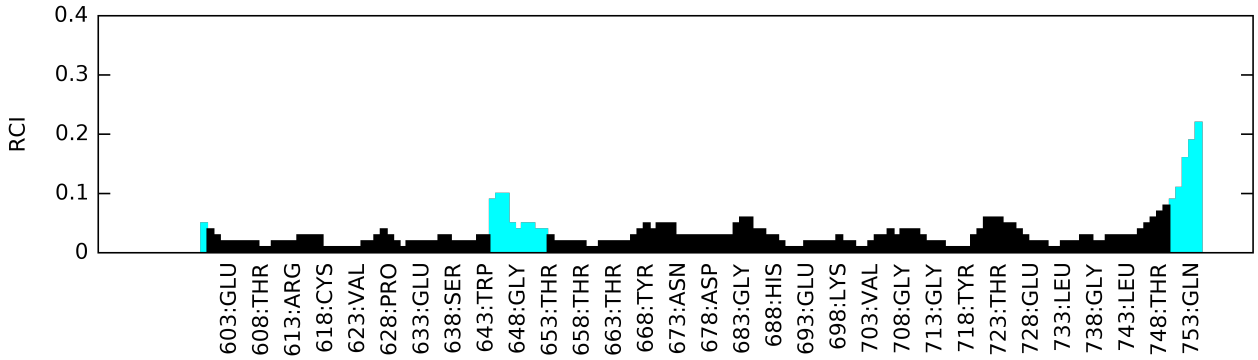
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	724	ARG	HD3	0.94	4.36 – 1.86	-8.7
1	A	720	ASN	HB3	0.59	4.41 – 1.11	-6.6
1	A	659	HIS	CE1	121.65	149.70 – 125.30	-6.5
1	A	625	ARG	HG2	-0.15	2.92 – 0.22	-6.4
1	A	724	ARG	HG3	-0.29	3.00 – 0.10	-6.4
1	A	625	ARG	HG3	-0.15	3.00 – 0.10	-5.9
1	A	691	ILE	HG21	-0.79	2.13 – -0.57	-5.8
1	A	691	ILE	HG22	-0.79	2.13 – -0.57	-5.8
1	A	691	ILE	HG23	-0.79	2.13 – -0.57	-5.8
1	A	667	ARG	HG2	0.13	2.92 – 0.22	-5.3

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:


RCI
0.4
0.3
0.2
0.1
0
603:GLU
608:THR
613:ARG
618:CYS
623:VAL
628:PRO
633:GLU
638:SER
643:TRP
648:GLY
653:THR
658:THR
663:THR
668:TYR
673:ASN
678:ASP
683:GLY
688:HIS
693:GLU
698:LYS
703:VAL
708:GLY
713:GLY
718:TYR
723:THR
728:GLU
733:LEU
738:GLY
743:LEU
748:THR
753:GLN