



Full wwPDB NMR Structure Validation Report ⓘ

Sep 14, 2020 – 02:57 PM BST

PDB ID : 6XFL
Title : Structural characterization of the type III secretion system pilotin-secretin complex InvH-InvG by NMR spectroscopy
Authors : Majewski, D.D.; Okon, M.; Heinkel, F.; Robb, C.S.; Vuckovic, M.; McIntosh, L.P.; Strynadka, N.C.J.
Deposited on : 2020-06-15

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 ⓘ symbol.

The following versions of software and data (see [references ⓘ](#)) 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)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	2.14.4.dev1
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.14.4.dev1

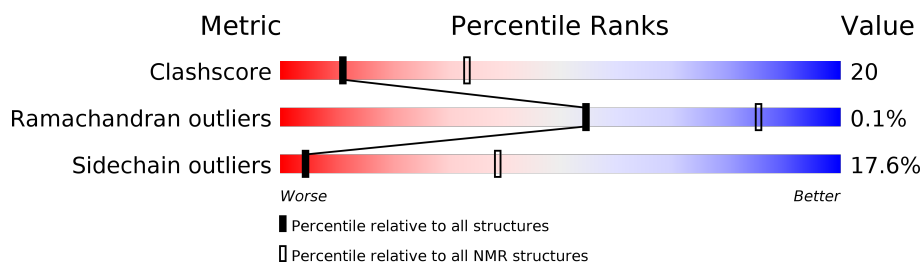
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

SOLUTION NMR

The overall completeness of chemical shifts assignment is 92%.

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 $\leq 5\%$

Mol	Chain	Length	Quality of chain
1	A	82	
2	B	47	

2 Ensemble composition and analysis

This entry contains 20 models. Model 1 is the overall representative, medoid model (most similar to other models).

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:85-A:146, B:543-B:561 (81)	0.19	1

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	2, 3, 5, 6, 7, 10, 11, 12, 13, 15, 17, 18, 19, 20
2	1, 4, 8
Single-model clusters	9; 14; 16

3 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 1987 atoms, of which 988 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Type 3 secretion system pilotin.

Mol	Chain	Residues	Atoms						Trace
1	A	79	Total	C	H	N	O	S	0
			1279	403	638	107	127	4	

There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	66	GLY	-	expression tag	UNP P0CL43
A	67	SER	-	expression tag	UNP P0CL43
A	68	HIS	-	expression tag	UNP P0CL43
A	69	MET	-	expression tag	UNP P0CL43

- Molecule 2 is a protein called Type 3 secretion system secretin.

Mol	Chain	Residues	Atoms						Trace
2	B	45	Total	C	H	N	O	S	0
			708	223	350	64	70	1	

There are 4 discrepancies between the modelled and reference sequences:

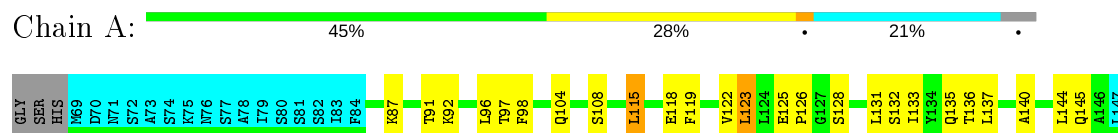
Chain	Residue	Modelled	Actual	Comment	Reference
B	516	GLY	-	expression tag	UNP P35672
B	517	SER	-	expression tag	UNP P35672
B	518	HIS	-	expression tag	UNP P35672
B	519	MET	-	expression tag	UNP P35672

4 Residue-property plots [i](#)

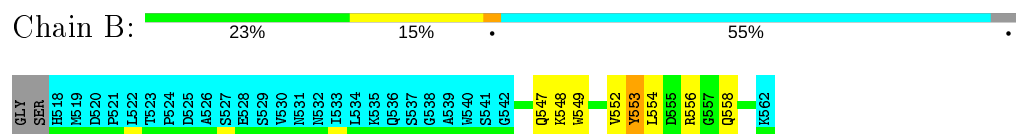
4.1 Average score per residue in the NMR ensemble

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: Type 3 secretion system pilotin



- Molecule 2: Type 3 secretion system secretin

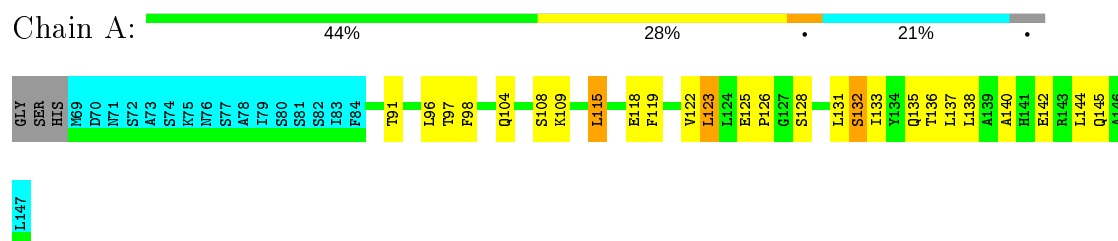


4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

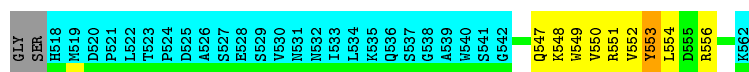
4.2.1 Score per residue for model 1 (medoid)

- Molecule 1: Type 3 secretion system pilotin



- Molecule 2: Type 3 secretion system secretin

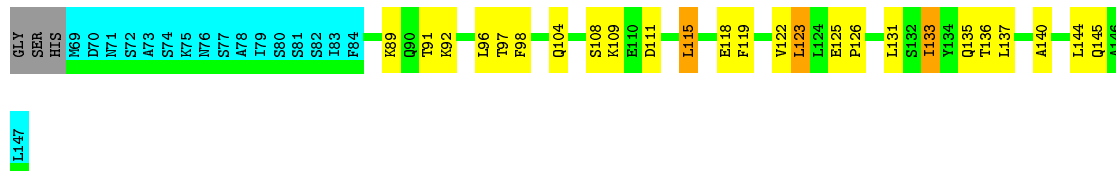




4.2.2 Score per residue for model 2

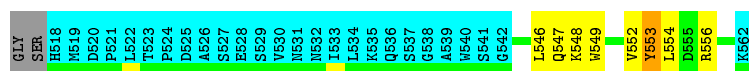
- Molecule 1: Type 3 secretion system pilotin

Chain A: 45% 27% 21%



- Molecule 2: Type 3 secretion system secretin

Chain B: 23% 15% 55%



4.2.3 Score per residue for model 3

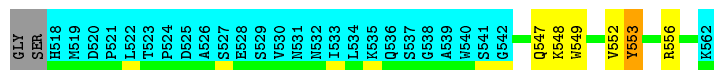
- Molecule 1: Type 3 secretion system pilotin

Chain A: 50% 24% 21%



- Molecule 2: Type 3 secretion system secretin

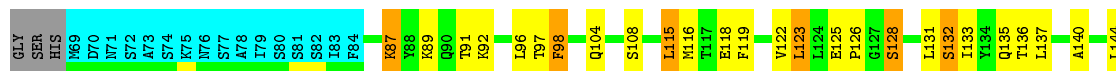
Chain B: 28% 11% 55%



4.2.4 Score per residue for model 4

- Molecule 1: Type 3 secretion system pilotin

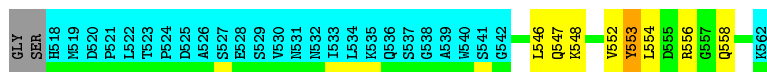
Chain A: 43% 26% 7% 21%





- Molecule 2: Type 3 secretion system secretin

Chain B: 23% 15% 55%



4.2.5 Score per residue for model 5

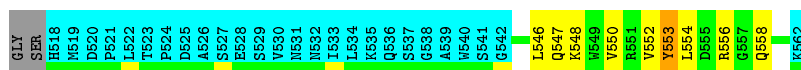
- Molecule 1: Type 3 secretion system pilotin

Chain A: 40% 33% 21%



- Molecule 2: Type 3 secretion system secretin

Chain B: 21% 17% 55%



4.2.6 Score per residue for model 6

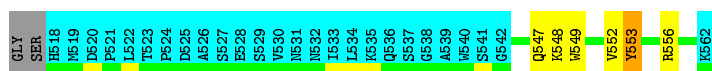
- Molecule 1: Type 3 secretion system pilotin

Chain A: 43% 28% 5% 21%



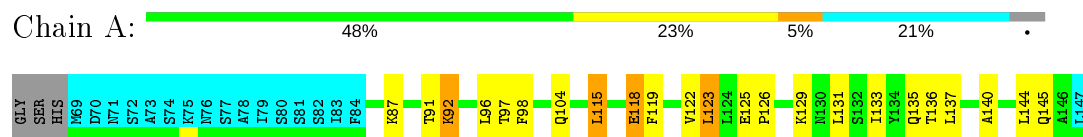
- Molecule 2: Type 3 secretion system secretin

Chain B: 28% 11% 55%

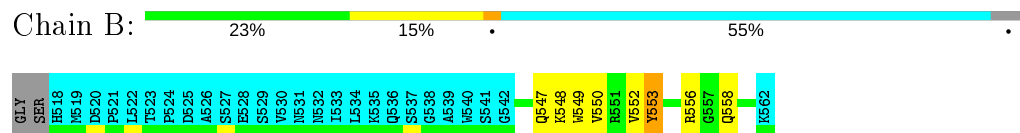


4.2.7 Score per residue for model 7

- Molecule 1: Type 3 secretion system pilotin

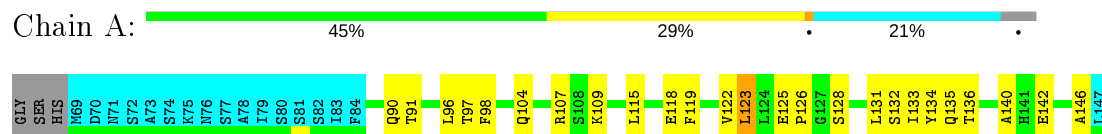


- Molecule 2: Type 3 secretion system secretin

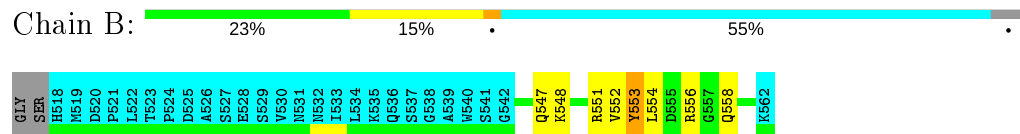


4.2.8 Score per residue for model 8

- Molecule 1: Type 3 secretion system pilotin

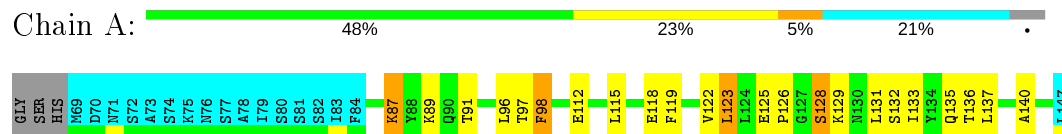


- Molecule 2: Type 3 secretion system secretin

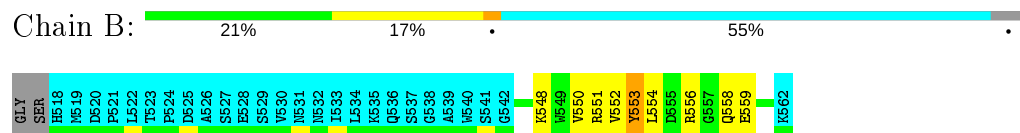


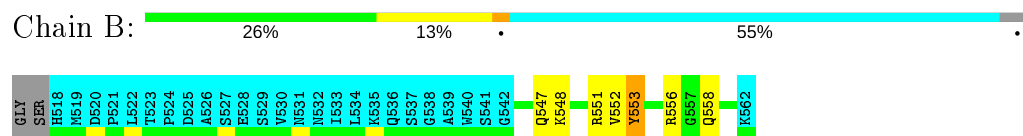
4.2.9 Score per residue for model 9

- Molecule 1: Type 3 secretion system pilotin



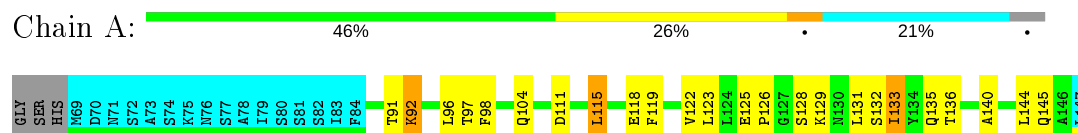
- Molecule 2: Type 3 secretion system secretin



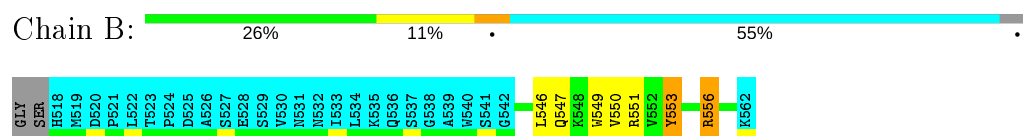


4.2.13 Score per residue for model 13

- Molecule 1: Type 3 secretion system pilotin

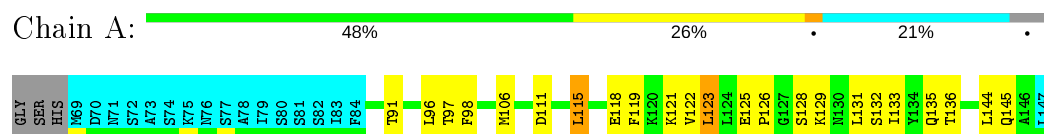


- Molecule 2: Type 3 secretion system secretin

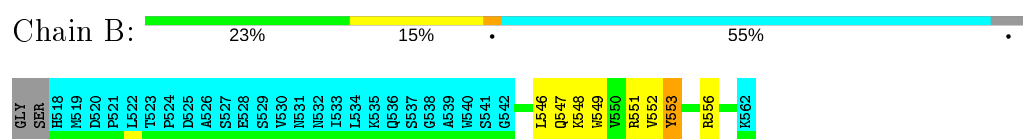


4.2.14 Score per residue for model 14

- Molecule 1: Type 3 secretion system pilotin

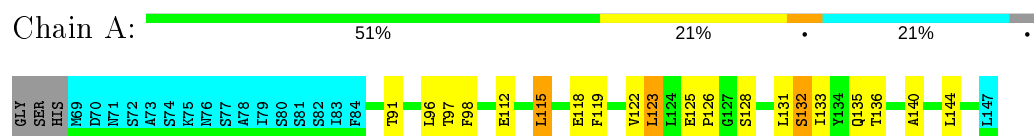


- Molecule 2: Type 3 secretion system secretin



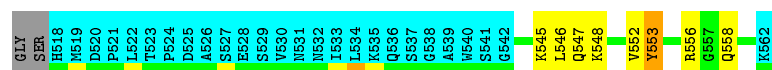
4.2.15 Score per residue for model 15

- Molecule 1: Type 3 secretion system pilotin



- Molecule 2: Type 3 secretion system secretin

Chain B: 



4.2.16 Score per residue for model 16

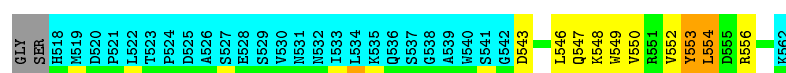
- Molecule 1: Type 3 secretion system pilotin

Chain A: 



- Molecule 2: Type 3 secretion system secretin

Chain B: 



4.2.17 Score per residue for model 17

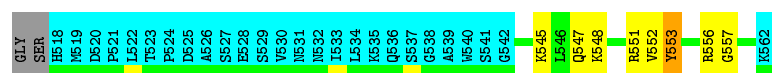
- Molecule 1: Type 3 secretion system pilotin

Chain A: 



- Molecule 2: Type 3 secretion system secretin

Chain B: 



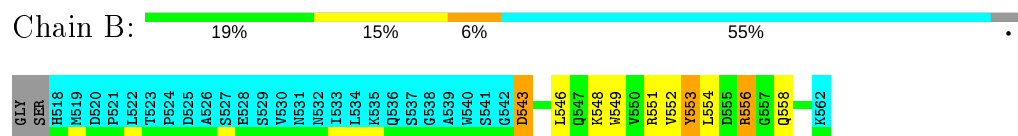
4.2.18 Score per residue for model 18

- Molecule 1: Type 3 secretion system pilotin

Chain A: 

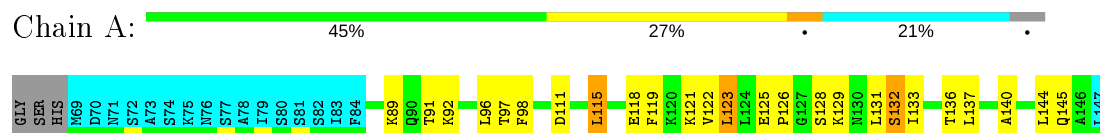


- Molecule 2: Type 3 secretion system secretin

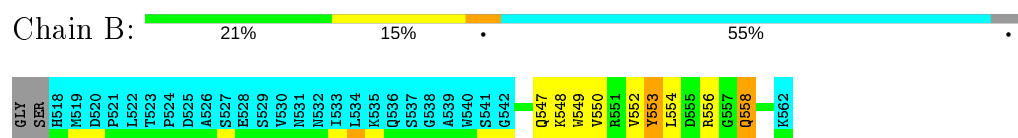


4.2.19 Score per residue for model 19

- Molecule 1: Type 3 secretion system pilotin

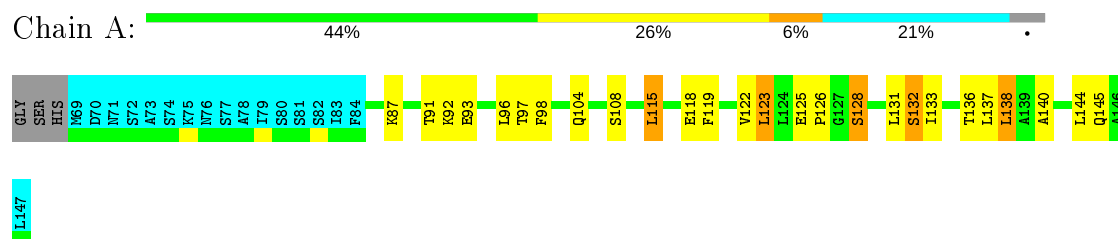


- Molecule 2: Type 3 secretion system secretin

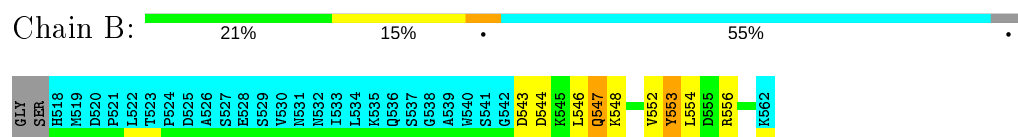


4.2.20 Score per residue for model 20

- Molecule 1: Type 3 secretion system pilotin



- Molecule 2: Type 3 secretion system secretin



5 Refinement protocol and experimental data overview

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

Of the 100 calculated structures, 20 were deposited, based on the following criterion: *target function*.

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

Software name	Classification	Version
CYANA	refinement	3.98.13
CYANA	structure calculation	3.98.13

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	1561
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	92%

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

6 Model quality

6.1 Standard geometry

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	519	518	516	26±3
2	B	164	164	164	7±1
All	All	13660	13640	13600	553

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 20.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:131:LEU:HD11	1:A:136:THR:HG23	0.98	1.33	17	14
1:A:119:PHE:O	1:A:123:LEU:HD23	0.75	1.82	13	1
1:A:91:THR:HG22	1:A:133:ILE:HG21	0.74	1.60	20	13
1:A:91:THR:HG21	2:B:553:TYR:CE2	0.70	2.22	13	20
1:A:133:ILE:HD12	2:B:553:TYR:CG	0.69	2.23	19	18
1:A:123:LEU:HD23	2:B:552:VAL:HG12	0.66	1.66	18	19
1:A:91:THR:HG21	2:B:553:TYR:CZ	0.66	2.26	12	20
1:A:98:PHE:CD2	1:A:137:LEU:HD21	0.65	2.27	6	4
1:A:123:LEU:HD12	2:B:556:ARG:CD	0.64	2.23	13	1
1:A:131:LEU:CD1	1:A:136:THR:HG23	0.63	2.24	11	14
1:A:118:GLU:O	1:A:122:VAL:HG13	0.62	1.94	16	20
1:A:104:GLN:OE1	1:A:115:LEU:HD12	0.62	1.95	12	4
2:B:548:LYS:O	2:B:552:VAL:HG23	0.61	1.95	19	19

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:91:THR:HB	1:A:133:ILE:HD13	0.61	1.72	16	14
1:A:115:LEU:HG	1:A:144:LEU:HD22	0.60	1.74	6	18
1:A:115:LEU:CG	1:A:144:LEU:HD22	0.60	2.27	6	15
1:A:115:LEU:CD1	1:A:144:LEU:HD22	0.58	2.28	14	9
1:A:115:LEU:HD12	1:A:144:LEU:HD22	0.58	1.74	14	8
2:B:543:ASP:O	2:B:546:LEU:HD23	0.58	1.98	18	1
1:A:104:GLN:NE2	1:A:115:LEU:HD12	0.58	2.14	5	1
1:A:119:PHE:CZ	1:A:123:LEU:HD21	0.58	2.32	15	19
1:A:92:LYS:HD2	2:B:550:VAL:HG21	0.57	1.74	5	3
1:A:131:LEU:HD12	1:A:132:SER:O	0.56	2.01	19	10
1:A:131:LEU:HD13	1:A:135:GLN:HB2	0.56	1.78	2	17
1:A:136:THR:HG21	2:B:556:ARG:NH1	0.55	2.17	18	1
1:A:111:ASP:OD2	1:A:144:LEU:HD21	0.55	2.00	10	5
1:A:104:GLN:NE2	1:A:115:LEU:HD13	0.55	2.16	16	1
1:A:119:PHE:O	1:A:123:LEU:HD22	0.54	2.03	6	19
1:A:131:LEU:HD11	1:A:136:THR:HG22	0.53	1.79	13	6
1:A:123:LEU:HD11	1:A:136:THR:HB	0.53	1.81	8	18
1:A:114:GLN:HG3	1:A:144:LEU:HD11	0.52	1.82	5	1
1:A:87:LYS:O	1:A:91:THR:HG23	0.52	2.04	9	6
1:A:119:PHE:O	1:A:123:LEU:HD13	0.52	2.04	20	19
1:A:104:GLN:HE22	1:A:115:LEU:HD12	0.52	1.64	5	1
1:A:144:LEU:HD23	1:A:145:GLN:HE21	0.52	1.65	5	15
1:A:96:LEU:HD12	1:A:97:THR:N	0.51	2.20	6	20
1:A:115:LEU:HA	1:A:144:LEU:HD13	0.50	1.83	10	4
1:A:91:THR:HG22	1:A:133:ILE:CG2	0.49	2.34	20	4
1:A:128:SER:O	1:A:131:LEU:HD23	0.49	2.08	12	18
1:A:115:LEU:HD22	2:B:549:TRP:CH2	0.48	2.43	13	11
2:B:550:VAL:HG12	2:B:554:LEU:HD23	0.48	1.83	1	4
1:A:119:PHE:CE2	1:A:137:LEU:HD23	0.48	2.43	11	6
2:B:546:LEU:HD12	2:B:547:GLN:N	0.48	2.23	16	3
1:A:122:VAL:HG21	1:A:140:ALA:HB2	0.48	1.85	8	17
1:A:125:GLU:CB	1:A:126:PRO:CD	0.46	2.94	16	20
1:A:122:VAL:HG23	1:A:123:LEU:HD13	0.46	1.87	5	11
1:A:131:LEU:HD11	1:A:136:THR:CG2	0.46	2.30	18	2
1:A:92:LYS:HE2	2:B:550:VAL:HG21	0.46	1.87	7	1
1:A:96:LEU:HB3	2:B:546:LEU:HD13	0.45	1.88	18	1
1:A:92:LYS:HG3	2:B:546:LEU:HD11	0.45	1.87	18	1
1:A:118:GLU:O	1:A:122:VAL:HG22	0.45	2.11	5	3
1:A:115:LEU:CA	1:A:144:LEU:HD13	0.45	2.42	10	1
1:A:96:LEU:HB3	2:B:546:LEU:HD22	0.45	1.88	13	5
1:A:125:GLU:N	1:A:126:PRO:HD2	0.45	2.27	19	20

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:122:VAL:O	1:A:126:PRO:CD	0.45	2.65	9	18
1:A:119:PHE:CE2	1:A:137:LEU:HD12	0.44	2.47	17	1
1:A:123:LEU:HD21	1:A:136:THR:HB	0.44	1.88	13	1
1:A:123:LEU:HD12	2:B:556:ARG:CG	0.43	2.43	13	1
1:A:92:LYS:CD	2:B:550:VAL:HG21	0.43	2.44	5	2
1:A:123:LEU:HD12	2:B:556:ARG:NE	0.43	2.27	13	1
1:A:142:GLU:O	1:A:146:ALA:HB2	0.42	2.14	8	1
1:A:114:GLN:CG	1:A:144:LEU:HD11	0.42	2.45	5	1
1:A:138:LEU:O	1:A:138:LEU:HD12	0.41	2.15	20	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	62/82 (76%)	58±1 (93±2%)	4±1 (7±2%)	0±0 (0±0%)	100	100
2	B	19/47 (40%)	16±1 (87±4%)	3±1 (13±4%)	0±0 (0±1%)	44	80
All	All	1620/2580 (63%)	1480 (91%)	139 (9%)	1 (0%)	54	85

All 1 unique Ramachandran outliers are listed below.

Mol	Chain	Res	Type	Models (Total)
2	B	557	GLY	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	57/74 (77%)	49±2 (85±3%)	8±2 (15±3%)	6	45

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
2	B	17/40 (42%)	12±1 (73±6%)	5±1 (27±6%)	2	21
All	All	1480/2280 (65%)	1219 (82%)	261 (18%)	4	39

All 38 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)
2	B	556	ARG	20
2	B	553	TYR	20
1	A	98	PHE	20
1	A	123	LEU	19
1	A	115	LEU	18
2	B	547	GLN	16
1	A	132	SER	14
1	A	108	SER	11
2	B	558	GLN	11
1	A	92	LYS	10
1	A	104	GLN	10
1	A	129	LYS	10
2	B	551	ARG	10
1	A	121	LYS	8
2	B	554	LEU	8
1	A	89	LYS	7
1	A	109	LYS	6
1	A	87	LYS	5
1	A	137	LEU	4
2	B	545	LYS	3
1	A	93	GLU	3
2	B	543	ASP	3
1	A	138	LEU	3
1	A	128	SER	3
1	A	106	MET	2
1	A	107	ARG	2
1	A	118	GLU	2
1	A	112	GLU	2
1	A	133	ILE	2
1	A	134	TYR	1
1	A	113	GLU	1
1	A	116	MET	1
1	A	94	GLN	1
2	B	559	GLU	1
2	B	544	ASP	1

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Mol	Chain	Res	Type	Models (Total)
1	A	142	GLU	1
1	A	111	ASP	1
1	A	90	GLN	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

The completeness of assignment taking into account all chemical shift lists is 92% for the well-defined parts and 91% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: 2020-06-04_HG_Shifts.star

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	1561
Number of shifts mapped to atoms	1561
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	1

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction \pm precision, ppm	Suggested action
$^{13}\text{C}_\alpha$	122	-0.59 ± 0.13	Should be applied
$^{13}\text{C}_\beta$	119	0.18 ± 0.08	None needed (< 0.5 ppm)
$^{13}\text{C}'$	117	-0.42 ± 0.08	None needed (< 0.5 ppm)
^{15}N	118	-0.14 ± 0.11	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 92%, i.e. 998 atoms were assigned a chemical shift out of a possible 1088. 0 out of 13 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^1H	^{13}C	^{15}N
Backbone	399/401 (100%)	160/160 (100%)	160/162 (99%)	79/79 (100%)
Sidechain	516/600 (86%)	326/354 (92%)	181/215 (84%)	9/31 (29%)

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	Total	¹ H	¹³ C	¹⁵ N
Aromatic	83/87 (95%)	45/45 (100%)	37/37 (100%)	1/5 (20%)
Overall	998/1088 (92%)	531/559 (95%)	378/414 (91%)	89/115 (77%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 91%, i.e. 1434 atoms were assigned a chemical shift out of a possible 1569. 0 out of 17 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	¹ H	¹³ C	¹⁵ N
Backbone	597/612 (98%)	240/244 (98%)	239/248 (96%)	118/120 (98%)
Sidechain	727/841 (86%)	458/497 (92%)	255/305 (84%)	14/39 (36%)
Aromatic	110/116 (95%)	60/60 (100%)	48/48 (100%)	2/8 (25%)
Overall	1434/1569 (91%)	758/801 (95%)	542/601 (90%)	134/167 (80%)

7.1.4 Statistically unusual chemical shifts ⓘ

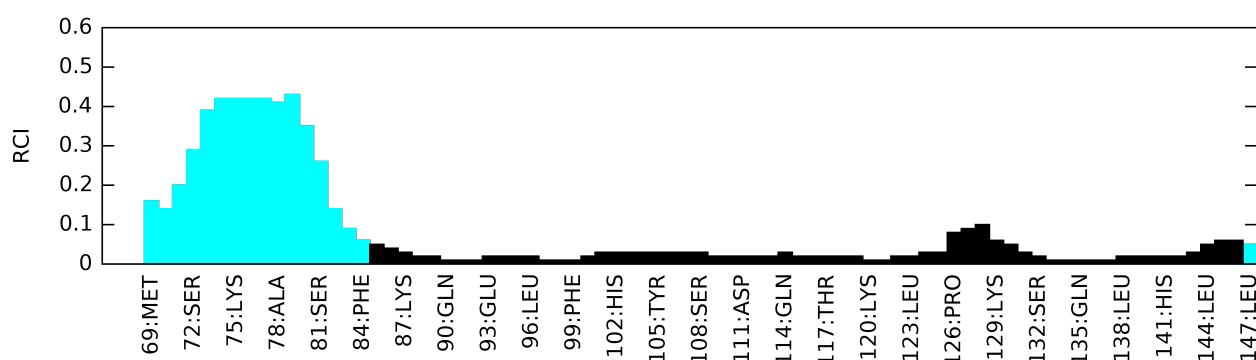
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	A	133	ILE	HB	0.32	3.24 – 0.34	-5.1

7.1.5 Random Coil Index (RCI) plots ⓘ

The images below report *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:



Random coil index (RCI) for chain B:

