Full wwPDB NMR Structure Validation Report (i)

Apr 26, 2016 – 02:29 PM BST

PDB ID	:	1FI3
Title	:	SOLUTION STRUCTURE OF THE M61H MUTANT OF PSEUDOMONAS STUTZERI SUBSTRAIN ZOBELL FERROCYTOCHROME C-551
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Deposited on	:	2000-08-03

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
http://wwpdb.org/validation/2016/NMRValidationReportHelp
with specific help available everywhere you see the (i) symbol.

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

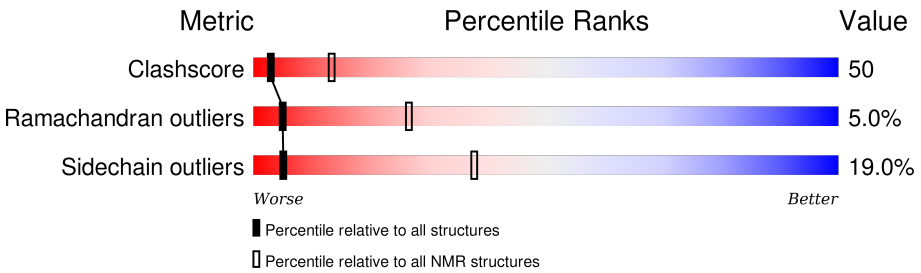
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	1.7.1 (RC1), CSD as537be (2016)
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	rb-20027457
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	rb-20027457

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 46%.

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	114402	11133
Ramachandran outliers	111179	9975
Sidechain outliers	111093	9958

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	82	40% 48% 12%

2 Ensemble composition and analysis (i)

This entry contains 1 models. Identification of well-defined residues and clustering analysis are not possible.

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 1282 atoms, of which 637 are hydrogens and 0 are deuteriums.

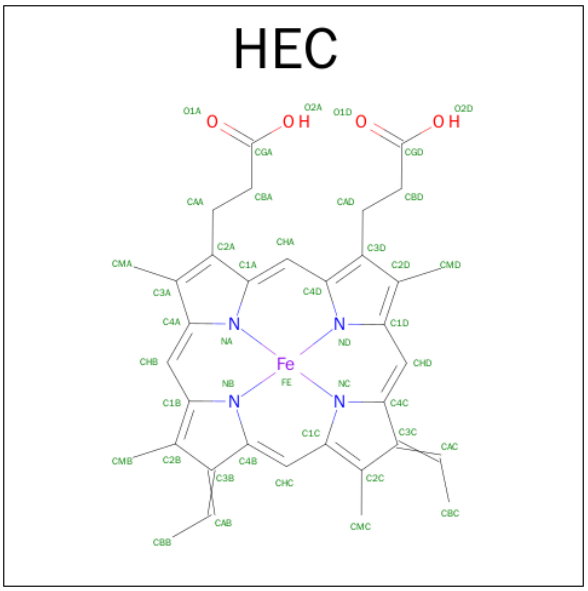
- Molecule 1 is a protein called CYTOCHROME C-551.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	82	1207	381	605	103	115	3	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	61	HIS	MET	ENGINEERED	UNP P00101

- Molecule 2 is HEME C (three-letter code: HEC) (formula: $C_{34}H_{34}FeN_4O_4$).



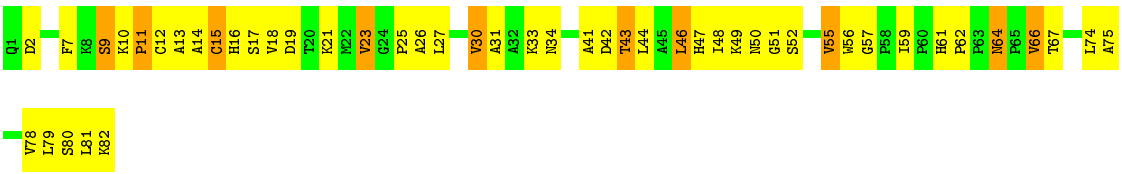
Mol	Chain	Residues	Atoms					
			Total	C	Fe	H	N	O
2	A	1	75	34	1	32	4	4

4 Residue-property plots (i)

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: CYTOCHROME C-551

Chain A: 40% 48% 12%



5 Refinement protocol and experimental data overview (i)

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

Of the ? calculated structures, 1 were deposited, based on the following criterion: *?*.

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

Software name	Classification	Version
CNSsolve	structure solution	0.9a
CNSsolve	refinement	0.9a

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)	BMRB entry 4960
Number of chemical shift lists	2
Total number of shifts	509
Number of shifts mapped to atoms	489
Number of unparsed shifts	0
Number of shifts with mapping errors	20
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	46%

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: HEC

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	602	605	605	62
2	A	43	32	30	16
All	All	645	637	635	64

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 50.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)
1:A:7:PHE:CE2	1:A:13:ALA:HB2	0.85	2.05
1:A:43:THR:HG22	1:A:47:HIS:CE1	0.73	2.18
1:A:48:ILE:HD11	2:A:83:HEC:C4A	0.71	2.16
1:A:7:PHE:CZ	1:A:13:ALA:HB2	0.69	2.23
1:A:61:HIS:ND1	1:A:62:PRO:HD2	0.69	2.03
1:A:74:LEU:O	1:A:78:VAL:HG13	0.68	1.89
1:A:79:LEU:HD12	1:A:80:SER:N	0.65	2.07
1:A:48:ILE:HG23	1:A:64:ASN:HB2	0.65	1.68
1:A:56:TRP:HE1	2:A:83:HEC:CGA	0.63	2.07
1:A:48:ILE:CD1	2:A:83:HEC:CHB	0.59	2.80
1:A:56:TRP:N	2:A:83:HEC:O1D	0.59	2.34
1:A:62:PRO:HG2	1:A:64:ASN:ND2	0.59	2.13

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Atom-1	Atom-2	Clash(Å)	Distance(Å)
1:A:79:LEU:HD12	1:A:79:LEU:C	0.59	2.18
1:A:48:ILE:HD11	2:A:83:HEC:CHB	0.59	2.28
1:A:23:VAL:O	2:A:83:HEC:HMD3	0.58	1.98
1:A:48:ILE:HG23	1:A:64:ASN:OD1	0.58	1.99
1:A:7:PHE:CZ	1:A:27:LEU:HD11	0.56	2.35
1:A:25:PRO:HG3	1:A:56:TRP:CD2	0.56	2.36
1:A:75:ALA:HA	1:A:78:VAL:CG2	0.56	2.30
1:A:48:ILE:CD1	2:A:83:HEC:C4A	0.56	2.83
1:A:46:LEU:O	1:A:50:ASN:HB2	0.54	2.03
1:A:34:ASN:ND2	1:A:44:LEU:HD21	0.53	2.18
1:A:15:CYS:CB	2:A:83:HEC:C2C	0.53	2.87
1:A:48:ILE:HG22	1:A:49:LYS:N	0.52	2.19
1:A:10:LYS:O	1:A:12:CYS:N	0.52	2.43
1:A:48:ILE:HG23	1:A:64:ASN:CB	0.51	2.34
1:A:25:PRO:HG3	1:A:56:TRP:CE3	0.50	2.40
1:A:75:ALA:HA	1:A:78:VAL:HG22	0.50	1.84
1:A:15:CYS:HB2	2:A:83:HEC:C2C	0.50	2.37
1:A:10:LYS:C	1:A:12:CYS:H	0.50	2.10
1:A:26:ALA:O	1:A:30:VAL:HG23	0.49	2.08
1:A:80:SER:O	1:A:82:LYS:N	0.48	2.47
1:A:13:ALA:O	1:A:16:HIS:O	0.48	2.32
1:A:75:ALA:CA	1:A:78:VAL:HG22	0.47	2.39
1:A:9:SER:O	1:A:11:PRO:HD3	0.47	2.09
1:A:56:TRP:NE1	2:A:83:HEC:CGA	0.46	2.77
1:A:46:LEU:HD12	1:A:47:HIS:CD2	0.46	2.46
1:A:42:ASP:O	1:A:46:LEU:HD23	0.45	2.11
1:A:55:VAL:HG21	2:A:83:HEC:O1A	0.45	2.12
1:A:74:LEU:O	1:A:78:VAL:HG22	0.45	2.11
1:A:62:PRO:O	1:A:64:ASN:ND2	0.45	2.49
1:A:66:VAL:CG2	1:A:67:THR:N	0.44	2.80
1:A:48:ILE:CA	1:A:64:ASN:OD1	0.44	2.66
1:A:7:PHE:CZ	1:A:13:ALA:CB	0.43	3.00
1:A:33:LYS:HG3	1:A:56:TRP:CH2	0.43	2.48
1:A:7:PHE:CE2	1:A:27:LEU:HD11	0.43	2.49
1:A:78:VAL:HG23	1:A:79:LEU:N	0.43	2.29
2:A:83:HEC:HMC1	2:A:83:HEC:HBC3	0.42	1.91
1:A:15:CYS:O	1:A:23:VAL:HG23	0.42	2.13
1:A:56:TRP:HB2	2:A:83:HEC:CAD	0.42	2.45
1:A:7:PHE:CE2	1:A:13:ALA:CB	0.42	2.94
1:A:59:ILE:O	2:A:83:HEC:HBD2	0.42	2.15
1:A:42:ASP:O	1:A:46:LEU:CD2	0.41	2.68
1:A:43:THR:O	1:A:47:HIS:CD2	0.41	2.73

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Atom-1	Atom-2	Clash(Å)	Distance(Å)
1:A:43:THR:O	1:A:47:HIS:CG	0.41	2.74
1:A:61:HIS:CG	1:A:62:PRO:HD2	0.41	2.50
1:A:57:GLY:O	2:A:83:HEC:O2D	0.41	2.39
1:A:41:ALA:HB2	1:A:75:ALA:HB1	0.41	1.91
1:A:18:VAL:HG13	1:A:19:ASP:N	0.41	2.30
2:A:83:HEC:HBA2	2:A:83:HEC:HHA	0.40	1.93
1:A:74:LEU:O	1:A:78:VAL:CG1	0.40	2.66
1:A:27:LEU:O	1:A:31:ALA:CB	0.40	2.69
1:A:11:PRO:O	1:A:14:ALA:N	0.40	2.55
1:A:75:ALA:O	1:A:79:LEU:HD23	0.40	2.16

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	80/82 (98%)	68 (85%)	8 (10%)	4 (5%)	5 26
All	All	80/82 (98%)	68 (85%)	8 (10%)	4 (5%)	5 26

All 4 Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type
1	A	51	GLY
1	A	21	LYS
1	A	11	PRO
1	A	81	LEU

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	63/63 (100%)	51 (81%)	12 (19%)	5 38
All	All	63/63 (100%)	51 (81%)	12 (19%)	5 38

All 12 residues with a non-rotameric sidechain are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type
1	A	46	LEU
1	A	17	SER
1	A	55	VAL
1	A	23	VAL
1	A	43	THR
1	A	9	SER
1	A	15	CYS
1	A	2	ASP
1	A	30	VAL
1	A	66	VAL
1	A	64	ASN
1	A	52	SER

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)

1 ligand is 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	HEC	A	83	1	24,50,50	1.38	0 (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	HEC	A	83	1	19,82,82	2.86	2 (10%)

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	HEC	A	83	1	-	0,6,54,54	0,0,8,8

There are no bond-length outliers.

All angle outliers are listed below. They are sorted according to the Z-score.

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	A	83	HEC	CBB-CAB-C3B	8.13	109.58	127.34
2	A	83	HEC	CBC-CAC-C3C	8.02	109.82	127.34

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

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 46% for the well-defined parts and 46% for the entire structure.

7.1 Chemical shift list 1

File name: BMRB entry 4960

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	489
Number of shifts mapped to atoms	489
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	14

7.1.2 Chemical shift referencing (i)

No chemical shift referencing corrections were calculated (not enough data).

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 46%, i.e. 430 atoms were assigned a chemical shift out of a possible 931. 0 out of 14 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}**H**	^{13}**C**	^{15}**N**
Backbone	144/396 (36%)	144/157 (92%)	0/164 (0%)	0/75 (0%)
Sidechain	262/481 (54%)	262/282 (93%)	0/186 (0%)	0/13 (0%)
Aromatic	24/54 (44%)	24/29 (83%)	0/20 (0%)	0/5 (0%)
Overall	430/931 (46%)	430/468 (92%)	0/370 (0%)	0/93 (0%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 46%, i.e. 430 atoms were assigned a chemical shift out of a possible 931. 0 out of 14 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	144/396 (36%)	144/157 (92%)	0/164 (0%)	0/75 (0%)
Sidechain	262/481 (54%)	262/282 (93%)	0/186 (0%)	0/13 (0%)
Aromatic	24/54 (44%)	24/29 (83%)	0/20 (0%)	0/5 (0%)
Overall	430/931 (46%)	430/468 (92%)	0/370 (0%)	0/93 (0%)

7.1.4 Statistically unusual chemical shifts (i)

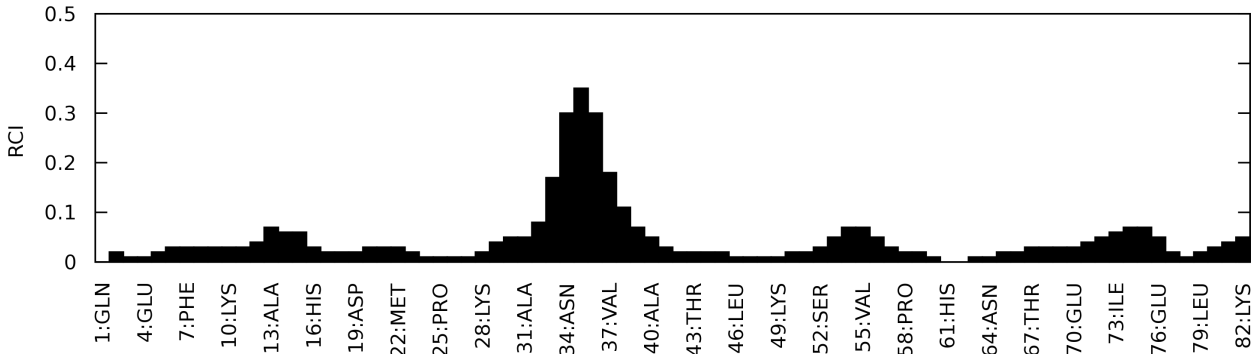
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	16	HIS	HD2	0.58	9.28 – 4.78	-14.3
1	A	16	HIS	HE1	0.69	10.53 – 5.43	-14.3
1	A	61	HIS	HB3	-1.23	5.00 – 1.10	-11.0
1	A	24	GLY	HA3	0.41	5.80 – 2.00	-9.2
1	A	62	PRO	HD3	0.23	5.52 – 1.72	-8.9
1	A	61	HIS	HE1	4.16	10.53 – 5.43	-7.5
1	A	61	HIS	HB2	0.45	4.91 – 1.31	-7.4
1	A	64	ASN	HD22	3.64	9.59 – 4.69	-7.1
1	A	25	PRO	HG2	-0.01	3.48 – 0.38	-6.3
1	A	16	HIS	HB2	1.13	4.91 – 1.31	-5.5
1	A	25	PRO	HG3	0.19	3.56 – 0.26	-5.2
1	A	27	LEU	HD23	-0.70	2.14 – -0.66	-5.1
1	A	27	LEU	HD22	-0.70	2.14 – -0.66	-5.1
1	A	27	LEU	HD21	-0.70	2.14 – -0.66	-5.1

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:



7.2 Chemical shift list 2

File name: BMRB entry 4960

Chemical shift list name: *assigned_chem_shift_list_2*

7.2.1 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	20
Number of shifts mapped to atoms	0
Number of unparsed shifts	0
Number of shifts with mapping errors	20
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	0

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

- Chain not found in structure. All 20 occurences are reported below.

Chain	Res	Type	Atom	Shift Data		
				Value	Uncertainty	Ambiguity
UNMAPPED	1	HEC	HAA2	4.23	0.02	1
UNMAPPED	1	HEC	HMC1	3.27	0.02	1
UNMAPPED	1	HEC	HAC	6.13	0.02	1
UNMAPPED	1	HEC	HAD2	3.84	0.02	1
UNMAPPED	1	HEC	HBB1	1.84	0.02	1
UNMAPPED	1	HEC	HHC	9.2	0.01	1
UNMAPPED	1	HEC	HMB1	3.62	0.02	1
UNMAPPED	1	HEC	HBA2	3.54	0.02	1
UNMAPPED	1	HEC	HBA1	2.85	0.02	1

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Chain	Res	Type	Atom	Shift Data		
				Value	Uncertainty	Ambiguity
UNMAPPED	1	HEC	HHB	9.2	0.01	1
UNMAPPED	1	HEC	HAB	5.88	0.02	1
UNMAPPED	1	HEC	HMD1	3.22	0.02	1
UNMAPPED	1	HEC	HAD1	4.35	0.02	1
UNMAPPED	1	HEC	HMA1	3.76	0.02	1
UNMAPPED	1	HEC	HBD2	2.6	0.02	1
UNMAPPED	1	HEC	HBC1	2.47	0.02	1
UNMAPPED	1	HEC	HAA1	4.6	0.02	1
UNMAPPED	1	HEC	HHA	9.55	0.01	1
UNMAPPED	1	HEC	HBD1	3.32	0.02	1
UNMAPPED	1	HEC	HHD	9.0	0.01	1

7.2.2 Chemical shift referencing (i)

No chemical shift referencing corrections were calculated (not enough data).

7.2.3 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 0%, i.e. 0 atoms were assigned a chemical shift out of a possible 931. 0 out of 14 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	0/396 (0%)	0/157 (0%)	0/164 (0%)	0/75 (0%)
Sidechain	0/481 (0%)	0/282 (0%)	0/186 (0%)	0/13 (0%)
Aromatic	0/54 (0%)	0/29 (0%)	0/20 (0%)	0/5 (0%)
Overall	0/931 (0%)	0/468 (0%)	0/370 (0%)	0/93 (0%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 0%, i.e. 0 atoms were assigned a chemical shift out of a possible 931. 0 out of 14 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	0/396 (0%)	0/157 (0%)	0/164 (0%)	0/75 (0%)
Sidechain	0/481 (0%)	0/282 (0%)	0/186 (0%)	0/13 (0%)
Aromatic	0/54 (0%)	0/29 (0%)	0/20 (0%)	0/5 (0%)
Overall	0/931 (0%)	0/468 (0%)	0/370 (0%)	0/93 (0%)

7.2.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

7.2.5 Random Coil Index (RCI) plots (i)

No *random coil index* (RCI) plot could be generated from the current chemical shift list (assigned_chem_shift_list_2). RCI is only applicable to proteins.