Full wwPDB NMR Structure Validation Report (i)

Jun 14, 2020 – 02:40 pm BST

PDB ID	:	2N2W
Title	:	Solution structure of [B26-B29 triazole cross-linked]-insulin analogue at pH 8.0
Authors	:	Veverka, V.; Hexnerova, R.; Jiracek, J.
Deposited on	:	2015-05-15

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 (i) symbol.

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

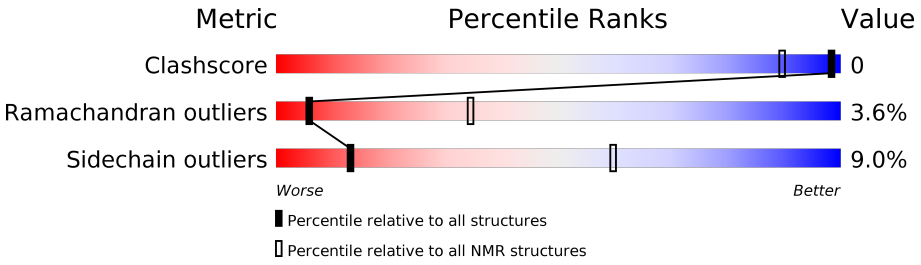
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	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 51%.

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	21	95% 5%
2	B	30	83% 17%

2 Ensemble composition and analysis

This entry contains 30 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: *closest to the average*.

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:2-A:21, B:1-B:25 (45)	0.54	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 5 clusters and 1 single-model cluster was found.

Cluster number	Models
1	3, 5, 10, 12, 13, 14, 15, 17, 25, 27
2	1, 2, 8, 19, 22, 23, 28, 30
3	4, 7, 9, 16, 26
4	18, 20, 24
5	6, 11, 21
Single-model clusters	29

3 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 775 atoms, of which 374 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Insulin A chain.

Mol	Chain	Residues	Atoms						Trace
1	A	21	Total	C	H	N	O	S	0
			312	99	149	25	35	4	

- Molecule 2 is a protein called Insulin B chain.

Mol	Chain	Residues	Atoms						Trace
2	B	30	Total	C	H	N	O	S	0
			463	153	225	42	41	2	

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	26	NVA	TYR	ENGINEERED MUTATION	UNP P01308
B	29	HIX	LYS	ENGINEERED MUTATION	UNP P01308

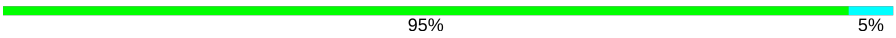
4 Residue-property plots

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.

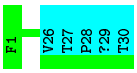
• Molecule 1: Insulin A chain

Chain A:



• Molecule 2: Insulin B chain

Chain B:



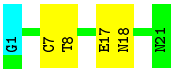
4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

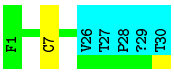
• Molecule 1: Insulin A chain

Chain A:



• Molecule 2: Insulin B chain

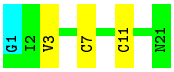
Chain B:



4.2.2 Score per residue for model 2

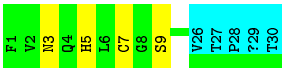
• Molecule 1: Insulin A chain

Chain A:



• Molecule 2: Insulin B chain

Chain B:



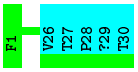
4.2.3 Score per residue for model 3

• Molecule 1: Insulin A chain

Chain A:

• Molecule 2: Insulin B chain

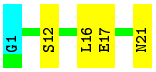
Chain B:



4.2.4 Score per residue for model 4

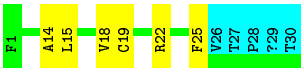
• Molecule 1: Insulin A chain

Chain A:



• Molecule 2: Insulin B chain

Chain B:



4.2.5 Score per residue for model 5 (medoid)

- Molecule 1: Insulin A chain

Chain A:

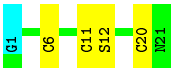
- Molecule 2: Insulin B chain

Chain B:

4.2.6 Score per residue for model 6

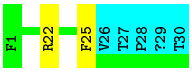
- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

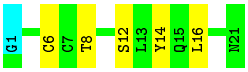
Chain B:



4.2.7 Score per residue for model 7

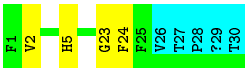
- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

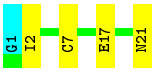
Chain B:



4.2.8 Score per residue for model 8

- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

Chain B:

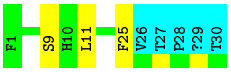
4.2.9 Score per residue for model 9

- Molecule 1: Insulin A chain

Chain A:

- Molecule 2: Insulin B chain

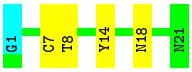
Chain B:



4.2.10 Score per residue for model 10

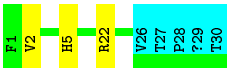
- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

Chain B:



4.2.11 Score per residue for model 11

- Molecule 1: Insulin A chain

Chain A:

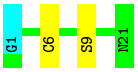
- Molecule 2: Insulin B chain

Chain B:

4.2.12 Score per residue for model 12

- Molecule 1: Insulin A chain

Chain A:



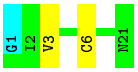
- Molecule 2: Insulin B chain

Chain B:

4.2.13 Score per residue for model 13

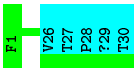
- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

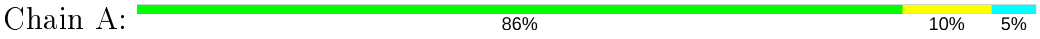
Chain B:



4.2.14 Score per residue for model 14

• Molecule 1: Insulin A chain

Chain A:



• Molecule 2: Insulin B chain

Chain B:

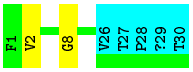
4.2.15 Score per residue for model 15

• Molecule 1: Insulin A chain

Chain A:

• Molecule 2: Insulin B chain

Chain B:



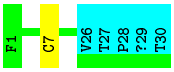
4.2.16 Score per residue for model 16

• Molecule 1: Insulin A chain

Chain A:

• Molecule 2: Insulin B chain

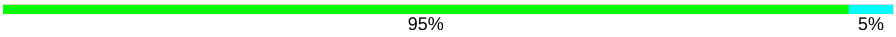
Chain B:



4.2.17 Score per residue for model 17

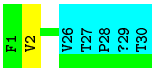
- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

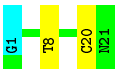
Chain B:



4.2.18 Score per residue for model 18

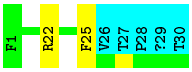
- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

Chain B:



4.2.19 Score per residue for model 19

- Molecule 1: Insulin A chain

Chain A:

- Molecule 2: Insulin B chain

Chain B:

4.2.20 Score per residue for model 20

- Molecule 1: Insulin A chain

Chain A:

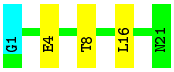
- Molecule 2: Insulin B chain

Chain B:

4.2.21 Score per residue for model 21

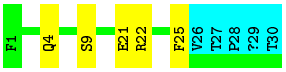
- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

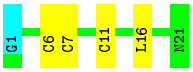
Chain B:



4.2.22 Score per residue for model 22

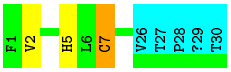
- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

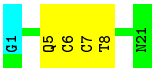
Chain B:



4.2.23 Score per residue for model 23

- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

Chain B:

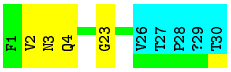
4.2.24 Score per residue for model 24

- Molecule 1: Insulin A chain

Chain A:

- Molecule 2: Insulin B chain

Chain B:



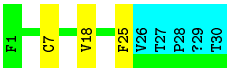
4.2.25 Score per residue for model 25

- Molecule 1: Insulin A chain

Chain A:

- Molecule 2: Insulin B chain

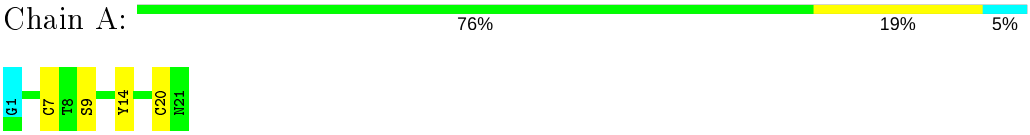
Chain B:



4.2.26 Score per residue for model 26

• Molecule 1: Insulin A chain

Chain A:



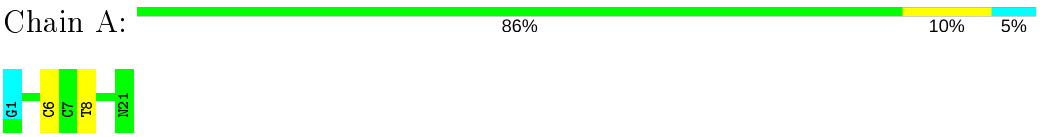
• Molecule 2: Insulin B chain

Chain B:

4.2.27 Score per residue for model 27

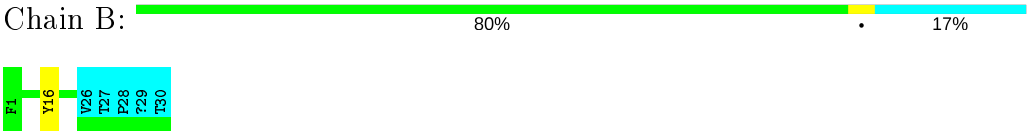
• Molecule 1: Insulin A chain

Chain A:



• Molecule 2: Insulin B chain

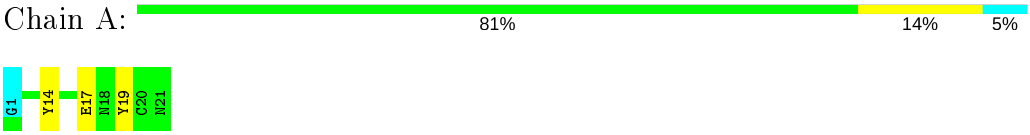
Chain B:



4.2.28 Score per residue for model 28

• Molecule 1: Insulin A chain

Chain A:



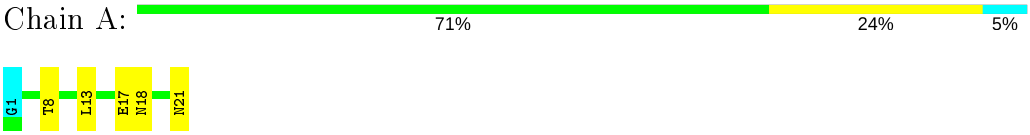
• Molecule 2: Insulin B chain

Chain B:

4.2.29 Score per residue for model 29

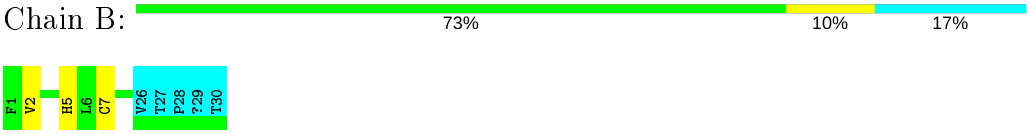
- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

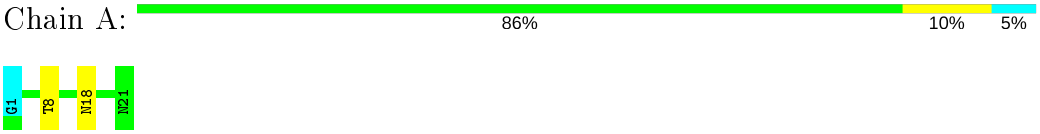
Chain B:



4.2.30 Score per residue for model 30

- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

Chain B:

5 Refinement protocol and experimental data overview

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

Of the 100 calculated structures, 30 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	structure solution	
YASARA	refinement	

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	371
Number of shifts mapped to atoms	371
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	51%

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: HIX, NVA

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	0.96±0.17	0±0/160 (0.1± 0.2%)	0.80±0.10	0±0/215 (0.0± 0.0%)
2	B	1.03±0.15	0±0/204 (0.0± 0.1%)	0.83±0.10	0±0/274 (0.1± 0.2%)
All	All	1.01	6/10920 (0.1%)	0.83	8/14670 (0.1%)

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
1	A	19	TYR	CD1-CE1	6.60	1.49	1.39	28	1
2	B	16	TYR	CE1-CZ	6.34	1.46	1.38	23	2
2	B	16	TYR	CG-CD2	5.91	1.46	1.39	30	1
1	A	14	TYR	CG-CD1	5.65	1.46	1.39	20	1
1	A	14	TYR	CD2-CE2	5.47	1.47	1.39	10	1

All 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	22	ARG	NE-CZ-NH1	6.63	123.61	120.30	18	6
2	B	22	ARG	NE-CZ-NH2	-6.28	117.16	120.30	19	2

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	159	144	144	0±0
2	B	199	189	189	0±0
All	All	10740	9990	9990	4

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 0.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:16:LEU:HD11	2:B:14:ALA:HB1	0.51	1.82	4	1
1:A:16:LEU:HD13	2:B:18:VAL:HG21	0.42	1.90	4	2
1:A:7:CYS:C	1:A:9:SER:H	0.42	2.18	26	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	19/21 (90%)	17±1 (89±4%)	2±1 (9±4%)	0±1 (2±3%)	12	54
2	B	24/30 (80%)	20±2 (82±9%)	3±2 (13±7%)	1±1 (5±5%)	4	24
All	All	1290/1530 (84%)	1094 (85%)	149 (12%)	47 (4%)	6	34

All 15 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
2	B	2	VAL	12
2	B	23	GLY	9
2	B	25	PHE	6
1	A	12	SER	4
1	A	9	SER	2
2	B	22	ARG	2

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Mol	Chain	Res	Type	Models (Total)
1	A	11	CYS	2
2	B	8	GLY	2
1	A	2	ILE	2
2	B	7	CYS	1
2	B	21	GLU	1
2	B	24	PHE	1
2	B	5	HIS	1
2	B	6	LEU	1
2	B	3	ASN	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	20/20 (100%)	18±1 (88±6%)	2±1 (12±6%)	9 52
2	B	21/24 (88%)	20±1 (93±5%)	1±1 (7±5%)	21 69
All	All	1230/1320 (93%)	1119 (91%)	111 (9%)	13 60

All 25 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	8	THR	13
2	B	7	CYS	10
1	A	7	CYS	10
2	B	5	HIS	9
1	A	6	CYS	8
1	A	18	ASN	7
2	B	4	GLN	7
1	A	17	GLU	6
1	A	21	ASN	4
2	B	9	SER	4
1	A	20	CYS	4
1	A	12	SER	3
1	A	16	LEU	3
2	B	11	LEU	3
1	A	11	CYS	3

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Mol	Chain	Res	Type	Models (Total)
1	A	14	TYR	3
2	B	25	PHE	3
1	A	3	VAL	2
1	A	5	GLN	2
2	B	3	ASN	2
2	B	6	LEU	1
2	B	19	CYS	1
1	A	13	LEU	1
2	B	15	LEU	1
1	A	4	GLU	1

6.3.3 RNA

There are no RNA molecules in this entry.

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

2 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	HIX	B	29	2	8,10,11	2.76±0.27	1±0 (12±0%)
2	NVA	B	26	2	5,6,7	1.56±0.46	0±0 (0±3%)

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	HIX	B	29	2	5,12,14	5.02±0.36	1±0 (20±0%)
2	NVA	B	26	2	2,6,8	0.30±0.13	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	HIX	B	29	2	-	0±0,4,6,8	0±0,1,1,1
2	NVA	B	26	2	-	0±0,4,5,7	-

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	B	29	HIX	ND1-NE1	8.25	1.47	1.34	10	30
2	B	26	NVA	CB-CA	5.29	1.60	1.53	27	1

All unique angle outliers are listed below.

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
2	B	29	HIX	NE2-NE1-ND1	12.13	96.14	111.24	26	30

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

6.5 Carbohydrates (i)

There are no carbohydrates in this entry.

6.6 Ligand geometry (i)

There are no ligands in this entry.

6.7 Other polymers

There are no such molecules in this entry.

6.8 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 51% for the well-defined parts and 52% 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	371
Number of shifts mapped to atoms	371
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	0

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	32	-0.94 ± 0.46	Should be applied
$^{13}C_{\beta}$	30	0.55 ± 0.26	Should be applied
$^{13}C'$	0	—	None (insufficient data)
^{15}N	0	—	None (insufficient data)

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 51%, i.e. 278 atoms were assigned a chemical shift out of a possible 540. 0 out of 10 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	97/225 (43%)	68/90 (76%)	29/90 (32%)	0/45 (0%)
Sidechain	152/250 (61%)	115/146 (79%)	37/95 (39%)	0/9 (0%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	29/65 (45%)	29/35 (83%)	0/28 (0%)	0/2 (0%)
Overall	278/540 (51%)	212/271 (78%)	66/213 (31%)	0/56 (0%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 52%, i.e. 298 atoms were assigned a chemical shift out of a possible 575. 0 out of 10 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	105/243 (43%)	73/97 (75%)	32/98 (33%)	0/48 (0%)
Sidechain	164/267 (61%)	123/156 (79%)	41/102 (40%)	0/9 (0%)
Aromatic	29/65 (45%)	29/35 (83%)	0/28 (0%)	0/2 (0%)
Overall	298/575 (52%)	225/288 (78%)	73/228 (32%)	0/59 (0%)

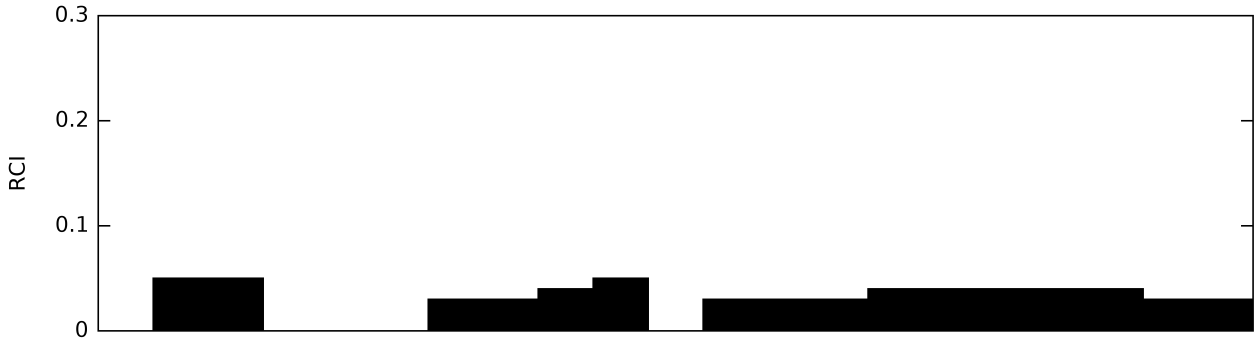
7.1.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

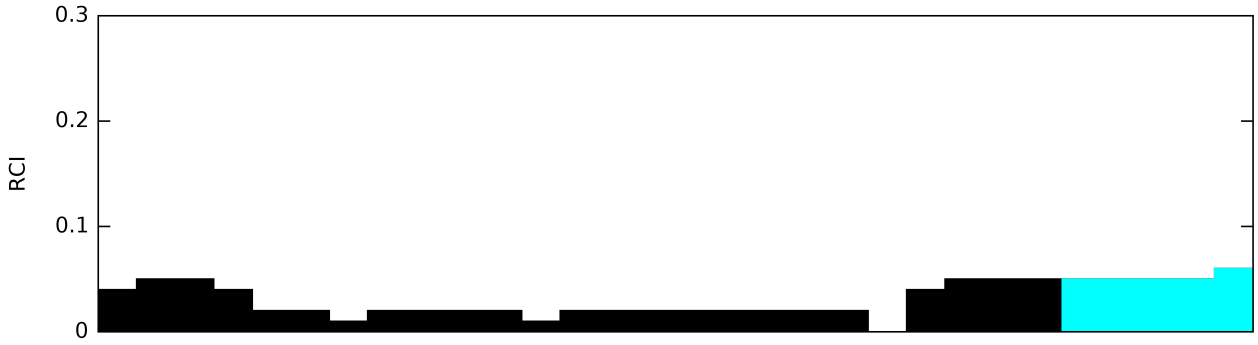
7.1.5 Random Coil Index (RCI) plots (i)

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:


0.3
0.2
0.1
0
RCI