Full wwPDB NMR Structure Validation Report (i)

Aug 20, 2020 – 08:55 AM BST

PDB ID	:	6KHA
Title	:	Solution structure of bovine insulin amyloid intermediate-2
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Deposited on	:	2019-07-14

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:

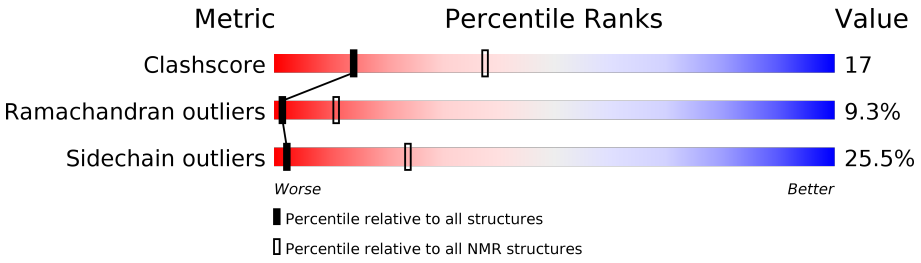
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	2.13
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.13

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 45%.

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	10% 57% 29% 5%
2	B	30	23% 20% 30% 17% 10%

2 Ensemble composition and analysis

This entry contains 10 models. Model 8 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:1-A:21, B:1-B:27 (48)	1.01	8

Ill-defined regions of proteins are excluded from the global statistics.

Ligands and non-protein polymers are included in the analysis.

NmrClust was unable to cluster the ensemble.

Error message: Inconsistent models

3 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 779 atoms, of which 379 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Insulin A chain.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	21	307	97	147	25	34	4	0

- Molecule 2 is a protein called Insulin B chain.

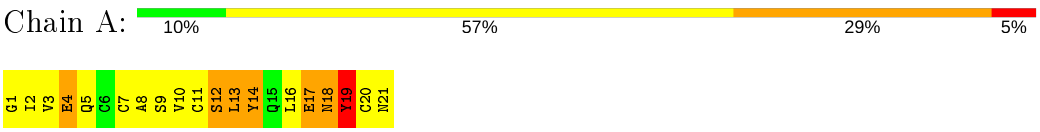
Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
2	B	30	472	157	232	40	41	2	0

4 Residue-property plots

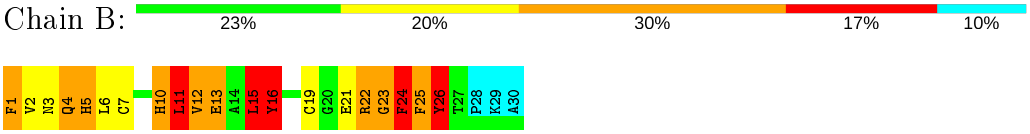
4.1 Average score per residue in the NMR ensemble

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: Insulin A chain



• Molecule 2: Insulin B chain



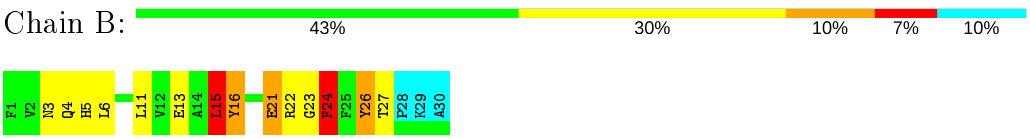
4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

• Molecule 1: Insulin A chain

• Molecule 2: Insulin B chain



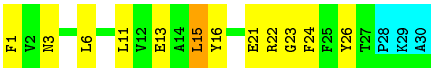
4.2.2 Score per residue for model 2

• Molecule 1: Insulin A chain

Chain A:

• Molecule 2: Insulin B chain

Chain B:



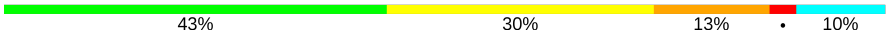
4.2.3 Score per residue for model 3

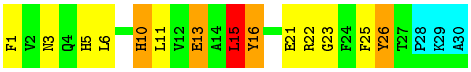
• Molecule 1: Insulin A chain

Chain A:

• Molecule 2: Insulin B chain

Chain B:

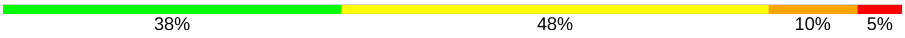




4.2.4 Score per residue for model 4

• Molecule 1: Insulin A chain

Chain A:



• Molecule 2: Insulin B chain

Chain B:

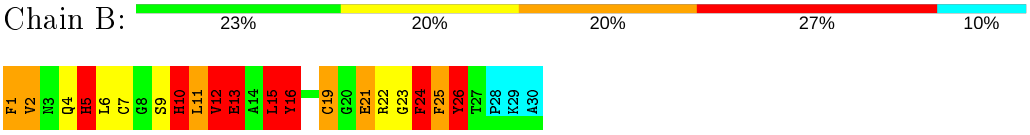
4.2.5 Score per residue for model 5

• Molecule 1: Insulin A chain

Chain A:

• Molecule 2: Insulin B chain

Chain B:



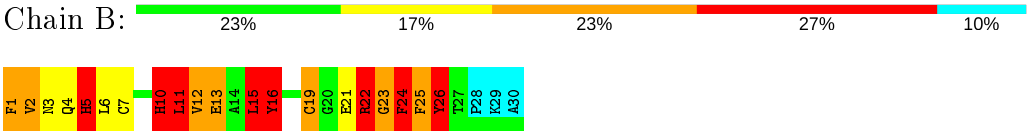
4.2.6 Score per residue for model 6

• Molecule 1: Insulin A chain

Chain A:

• Molecule 2: Insulin B chain

Chain B:



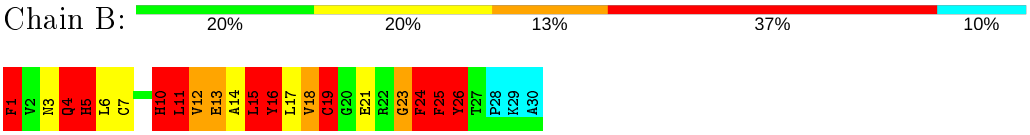
4.2.7 Score per residue for model 7

• Molecule 1: Insulin A chain

Chain A:

• Molecule 2: Insulin B chain

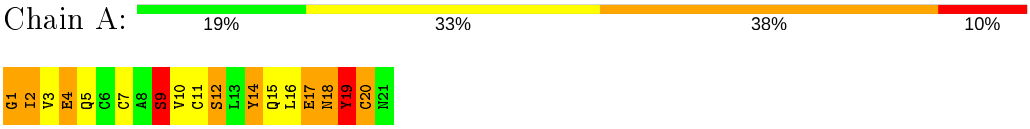
Chain B:



4.2.8 Score per residue for model 8 (medoid)

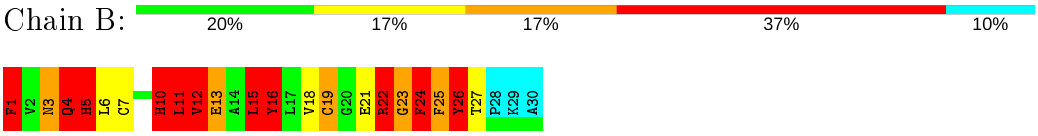
- Molecule 1: Insulin A chain

Chain A:



- Molecule 2: Insulin B chain

Chain B:



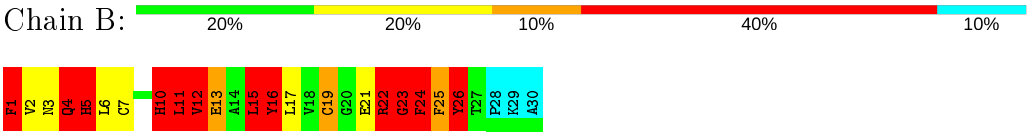
4.2.9 Score per residue for model 9

- Molecule 1: Insulin A chain

Chain A:

- Molecule 2: Insulin B chain

Chain B:



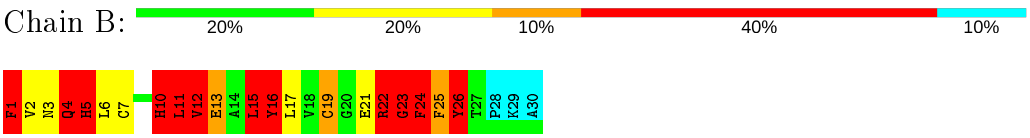
4.2.10 Score per residue for model 10

- Molecule 1: Insulin A chain

Chain A:

- Molecule 2: Insulin B chain

Chain B:



5 Refinement protocol and experimental data overview

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

Of the 50 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
Amber	refinement	
Amber	structure calculation	

The following table shows chemical shift validation statistics as aggregates over all chemical shift files. Detailed validation can be found in section 7 of this report.

Chemical shift file(s)	working_cs.cif
Number of chemical shift lists	1
Total number of shifts	308
Number of shifts mapped to atoms	308
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	45%

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

6 Model quality

6.1 Standard geometry

The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 5$ is considered an outlier worth inspection. RMSZ is the (average) root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	#Z>5	RMSZ	#Z>5
1	A	1.53±0.13	2±0/161 (1.3± 0.2%)	3.48±1.05	16±10/216 (7.5± 4.6%)
2	B	1.39±0.11	2±0/225 (0.9± 0.0%)	3.63±1.26	27±17/306 (8.8± 5.7%)
All	All	1.45	41/3860 (1.1%)	3.76	430/5220 (8.2%)

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand. A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	Chirality	Planarity
1	A	0.0±0.0	7.2±3.4
2	B	0.0±0.0	13.0±5.0
All	All	0	202

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	17	GLU	CD-OE2	11.15	1.38	1.25	3	10
2	B	21	GLU	CD-OE2	10.88	1.37	1.25	4	10
1	A	4	GLU	CD-OE2	10.70	1.37	1.25	8	10
2	B	13	GLU	CD-OE2	10.20	1.36	1.25	8	10
1	A	19	TYR	CB-CG	-5.06	1.44	1.51	8	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	16	TYR	CB-CG-CD1	-30.54	102.68	121.00	4	8
1	A	14	TYR	CB-CG-CD1	-28.89	103.67	121.00	8	9
1	A	14	TYR	CB-CG-CD2	-26.34	105.20	121.00	6	7

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Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
1	A	19	TYR	CB-CG-CD2	-25.72	105.57	121.00	4	10
2	B	26	TYR	CB-CG-CD2	-25.17	105.90	121.00	9	8
2	B	24	PHE	CB-CG-CD2	-22.66	104.94	120.80	8	8
2	B	25	PHE	CB-CG-CD1	-21.53	105.73	120.80	7	7
2	B	16	TYR	CB-CG-CD2	-20.64	108.61	121.00	7	7
2	B	1	PHE	CB-CG-CD2	-19.52	107.13	120.80	9	7
1	A	19	TYR	CG-CD2-CE2	-19.42	105.76	121.30	9	6
2	B	26	TYR	CB-CG-CD1	-18.65	109.81	121.00	8	8
2	B	26	TYR	CG-CD2-CE2	-17.39	107.39	121.30	5	4
1	A	14	TYR	CG-CD2-CE2	-16.97	107.72	121.30	8	6
2	B	26	TYR	CG-CD1-CE1	-15.65	108.78	121.30	8	6
2	B	24	PHE	CB-CG-CD1	-14.75	110.47	120.80	9	6
1	A	19	TYR	CD1-CE1-CZ	-14.33	106.90	119.80	9	4
1	A	19	TYR	CB-CG-CD1	-14.23	112.46	121.00	5	3
2	B	25	PHE	CB-CG-CD2	-13.43	111.40	120.80	7	6
2	B	16	TYR	CG-CD1-CE1	-11.63	112.00	121.30	4	7
1	A	19	TYR	CZ-CE2-CD2	-11.47	109.47	119.80	8	3
2	B	16	TYR	CG-CD2-CE2	-10.22	113.12	121.30	7	6
2	B	24	PHE	CG-CD2-CE2	-10.21	109.57	120.80	8	6
1	A	19	TYR	CG-CD1-CE1	-10.08	113.24	121.30	8	6
2	B	12	VAL	CG1-CB-CG2	9.89	126.72	110.90	7	7
1	A	14	TYR	N-CA-CB	-9.39	93.70	110.60	8	9
2	B	15	LEU	CB-CA-C	9.36	127.98	110.20	7	9
2	B	25	PHE	N-CA-CB	-9.18	94.08	110.60	8	7
1	A	10	VAL	CG1-CB-CG2	8.34	124.25	110.90	8	2
2	B	10	HIS	N-CA-CB	-8.21	95.83	110.60	8	8
2	B	10	HIS	CA-C-N	-8.18	99.21	117.20	9	6
2	B	12	VAL	CA-CB-CG1	7.71	122.46	110.90	8	6
1	A	12	SER	N-CA-CB	-7.61	99.09	110.50	9	6
1	A	14	TYR	CA-CB-CG	7.50	127.65	113.40	8	6
1	A	20	CYS	N-CA-CB	-7.41	97.27	110.60	8	6
1	A	14	TYR	CA-C-N	-7.37	101.00	117.20	8	3
1	A	14	TYR	CG-CD1-CE1	-7.29	115.47	121.30	8	6
1	A	12	SER	C-N-CA	7.25	139.82	121.70	6	4
2	B	13	GLU	N-CA-CB	-7.08	97.85	110.60	8	1
2	B	16	TYR	CA-CB-CG	7.07	126.83	113.40	7	3
2	B	25	PHE	CA-C-N	-7.01	101.78	117.20	9	5
1	A	19	TYR	N-CA-CB	-6.96	98.07	110.60	5	7
1	A	16	LEU	N-CA-CB	-6.95	96.50	110.40	8	6
1	A	18	ASN	N-CA-CB	-6.81	98.34	110.60	5	4
2	B	22	ARG	N-CA-CB	-6.75	98.46	110.60	9	5

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Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
2	B	25	PHE	CG-CD1-CE1	-6.70	113.44	120.80	9	6
2	B	5	HIS	CB-CG-CD2	-6.61	110.31	130.80	8	7
2	B	1	PHE	CG-CD2-CE2	-6.48	113.68	120.80	6	6
1	A	18	ASN	CA-C-N	-6.46	102.99	117.20	7	3
2	B	21	GLU	C-N-CA	6.41	137.73	121.70	9	5
2	B	2	VAL	CA-CB-CG1	6.40	120.49	110.90	5	5
2	B	11	LEU	N-CA-C	6.37	128.19	111.00	9	5
2	B	21	GLU	N-CA-CB	-6.35	99.17	110.60	9	7
2	B	7	CYS	N-CA-CB	-6.32	99.22	110.60	8	7
2	B	10	HIS	CB-CG-CD2	-6.28	111.34	130.80	9	5
2	B	18	VAL	CA-CB-CG1	6.26	120.29	110.90	7	2
2	B	5	HIS	CA-C-N	-6.26	103.43	117.20	7	2
2	B	24	PHE	CG-CD1-CE1	-6.19	114.00	120.80	8	4
2	B	21	GLU	CA-C-N	-6.14	103.70	117.20	9	4
1	A	14	TYR	N-CA-C	6.11	127.50	111.00	9	4
2	B	4	GLN	CA-C-N	-6.11	103.76	117.20	7	4
2	B	22	ARG	NE-CZ-NH1	6.11	123.36	120.30	8	1
2	B	1	PHE	CB-CG-CD1	6.05	125.04	120.80	4	1
1	A	19	TYR	CA-C-N	-6.04	103.91	117.20	9	4
2	B	17	LEU	CB-CA-C	6.03	121.65	110.20	9	2
2	B	26	TYR	CA-CB-CG	6.00	124.81	113.40	9	3
2	B	13	GLU	CA-C-N	-5.98	104.04	117.20	8	6
1	A	13	LEU	N-CA-CB	-5.97	98.47	110.40	9	3
1	A	2	ILE	CA-CB-CG2	5.95	122.81	110.90	9	2
2	B	23	GLY	CA-C-N	-5.92	104.18	117.20	8	5
2	B	18	VAL	CG1-CB-CG2	5.80	120.19	110.90	7	1
2	B	11	LEU	N-CA-CB	-5.79	98.81	110.40	9	4
2	B	3	ASN	C-N-CA	5.72	135.99	121.70	6	3
2	B	10	HIS	O-C-N	5.71	131.84	122.70	7	4
2	B	24	PHE	CD1-CG-CD2	-5.71	110.88	118.30	7	1
1	A	16	LEU	CB-CA-C	5.69	121.01	110.20	8	3
1	A	11	CYS	C-N-CA	5.68	135.90	121.70	8	3
1	A	8	ALA	N-CA-CB	-5.65	102.19	110.10	5	1
2	B	22	ARG	NE-CZ-NH2	-5.65	117.47	120.30	8	1
1	A	4	GLU	CA-C-N	-5.57	104.94	117.20	7	5
2	B	25	PHE	CD1-CG-CD2	5.54	125.50	118.30	7	1
1	A	3	VAL	CA-C-N	-5.51	105.08	117.20	5	1
1	A	17	GLU	CB-CA-C	-5.49	99.43	110.40	5	1
2	B	15	LEU	CB-CG-CD2	-5.48	101.68	111.00	9	3
2	B	16	TYR	N-CA-CB	-5.46	100.76	110.60	5	1
1	A	21	ASN	N-CA-CB	-5.42	100.84	110.60	7	3

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Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
1	A	20	CYS	CA-CB-SG	5.38	123.69	114.00	7	1
1	A	7	CYS	N-CA-CB	-5.34	100.99	110.60	7	1
2	B	9	SER	CA-C-N	-5.33	105.48	117.20	5	1
2	B	5	HIS	CB-CG-ND1	-5.33	109.88	123.20	9	2
2	B	4	GLN	CB-CA-C	5.30	121.00	110.40	7	1
1	A	18	ASN	CB-CA-C	5.29	120.98	110.40	9	2
1	A	9	SER	N-CA-CB	-5.28	102.58	110.50	9	3
2	B	25	PHE	O-C-N	5.25	131.10	122.70	9	2
2	B	26	TYR	N-CA-C	5.21	125.07	111.00	9	2
1	A	4	GLU	N-CA-CB	-5.15	101.33	110.60	6	2
1	A	14	TYR	CZ-CE2-CD2	-5.13	115.18	119.80	7	3
1	A	14	TYR	CD1-CG-CD2	5.12	123.53	117.90	4	1
2	B	10	HIS	N-CA-C	5.09	124.75	111.00	7	1
1	A	15	GLN	N-CA-CB	-5.08	101.46	110.60	8	1
2	B	10	HIS	CB-CG-ND1	5.07	135.88	123.20	9	2
1	A	19	TYR	C-N-CA	5.03	134.27	121.70	7	1
2	B	21	GLU	N-CA-C	5.02	124.55	111.00	5	1

There are no chirality outliers.

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

Mol	Chain	Res	Type	Group	Models (Total)
2	B	23	GLY	Peptide	10
1	A	14	TYR	Sidechain,Peptide	10
2	B	16	TYR	Sidechain	10
1	A	19	TYR	Sidechain	10
2	B	26	TYR	Sidechain,Peptide	9
2	B	5	HIS	Sidechain,Peptide	9
2	B	10	HIS	Peptide	8
2	B	13	GLU	Peptide	8
2	B	25	PHE	Sidechain,Peptide	8
1	A	1	GLY	Peptide	8
2	B	4	GLN	Peptide	8
2	B	24	PHE	Sidechain,Peptide	8
1	A	7	CYS	Peptide	7
2	B	11	LEU	Peptide,Mainchain	7
1	A	3	VAL	Peptide	7
1	A	4	GLU	Peptide	7
1	A	9	SER	Peptide	7
2	B	1	PHE	Sidechain	7

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Mol	Chain	Res	Type	Group	Models (Total)
2	B	19	CYS	Peptide	6
2	B	22	ARG	Peptide	4
2	B	3	ASN	Peptide	4
1	A	17	GLU	Peptide	3
1	A	8	ALA	Peptide	2
1	A	11	CYS	Peptide	2
1	A	12	SER	Peptide	2
2	B	2	VAL	Peptide	2
2	B	14	ALA	Peptide	1
2	B	21	GLU	Peptide	1
1	A	2	ILE	Peptide	1

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	160	147	145	6±5
2	B	218	207	205	8±6
All	All	3780	3540	3500	127

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 17.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:2:ILE:HD13	1:A:19:TYR:CD2	0.95	1.97	6	6
1:A:2:ILE:HD13	1:A:19:TYR:CG	0.80	2.12	8	5
2:B:12:VAL:HA	2:B:15:LEU:HD12	0.77	1.54	7	7
2:B:15:LEU:C	2:B:15:LEU:HD22	0.74	2.01	7	5
2:B:15:LEU:HD21	2:B:24:PHE:CE2	0.71	2.21	7	1
1:A:1:GLY:HA2	1:A:19:TYR:CD2	0.67	2.24	9	5
2:B:19:CYS:HB3	2:B:24:PHE:CD2	0.65	2.26	8	4
1:A:1:GLY:CA	1:A:19:TYR:CD2	0.63	2.81	9	4
2:B:24:PHE:HA	2:B:24:PHE:CE1	0.61	2.29	7	1
1:A:1:GLY:HA2	1:A:19:TYR:HD2	0.56	1.55	9	2
2:B:15:LEU:HD13	2:B:16:TYR:N	0.56	2.16	4	2

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:2:ILE:CD1	1:A:19:TYR:CG	0.55	2.89	7	3
1:A:1:GLY:HA2	1:A:19:TYR:CE2	0.54	2.38	5	1
2:B:15:LEU:HD11	2:B:24:PHE:CB	0.53	2.33	1	1
2:B:16:TYR:HA	2:B:24:PHE:CE2	0.53	2.39	8	5
2:B:12:VAL:HA	2:B:15:LEU:CD1	0.52	2.33	7	1
1:A:2:ILE:HG21	2:B:26:TYR:CE1	0.52	2.39	9	2
1:A:1:GLY:HA3	1:A:19:TYR:CE2	0.51	2.40	4	1
1:A:1:GLY:HA3	1:A:19:TYR:CZ	0.51	2.40	6	3
2:B:12:VAL:O	2:B:16:TYR:HB2	0.51	2.04	9	5
1:A:2:ILE:CG2	2:B:26:TYR:CE1	0.51	2.94	9	3
1:A:17:GLU:HB2	1:A:18:ASN:HB2	0.50	1.83	9	2
2:B:1:PHE:CE2	2:B:1:PHE:HA	0.50	2.42	8	3
1:A:20:CYS:SG	2:B:24:PHE:CE1	0.49	3.05	7	1
1:A:13:LEU:N	1:A:13:LEU:HD13	0.49	2.22	6	2
2:B:15:LEU:CD2	2:B:24:PHE:CE2	0.48	2.95	7	1
1:A:20:CYS:SG	2:B:24:PHE:HE1	0.48	2.31	7	1
1:A:2:ILE:HB	1:A:19:TYR:CE1	0.48	2.44	6	2
1:A:2:ILE:HD13	1:A:19:TYR:HD2	0.47	1.58	6	2
2:B:16:TYR:HB3	2:B:17:LEU:HG	0.47	1.85	7	1
1:A:13:LEU:O	1:A:17:GLU:HG3	0.47	2.09	9	3
1:A:18:ASN:HB3	1:A:19:TYR:CE2	0.47	2.45	7	2
2:B:19:CYS:SG	2:B:24:PHE:CE1	0.46	3.08	7	1
1:A:15:GLN:HB2	1:A:16:LEU:HG	0.46	1.87	9	2
2:B:19:CYS:HB3	2:B:24:PHE:CE2	0.46	2.45	5	2
1:A:20:CYS:SG	2:B:24:PHE:CE2	0.46	3.09	5	1
2:B:24:PHE:HA	2:B:24:PHE:CE2	0.46	2.45	5	1
2:B:24:PHE:CE1	2:B:24:PHE:CA	0.45	2.99	7	1
2:B:12:VAL:CA	2:B:15:LEU:HD12	0.45	2.36	7	3
1:A:2:ILE:HG21	2:B:25:PHE:CD2	0.45	2.46	7	1
2:B:11:LEU:H	2:B:11:LEU:HD13	0.45	1.71	7	1
2:B:24:PHE:N	2:B:24:PHE:CD1	0.45	2.85	5	1
1:A:2:ILE:HB	1:A:19:TYR:CD1	0.44	2.47	6	2
2:B:11:LEU:HD13	2:B:11:LEU:H	0.44	1.72	9	2
2:B:15:LEU:HD11	2:B:24:PHE:HB3	0.44	1.89	1	1
2:B:1:PHE:HA	2:B:1:PHE:CE2	0.44	2.44	7	1
1:A:19:TYR:CE2	1:A:19:TYR:CA	0.44	3.01	4	1
2:B:24:PHE:CZ	2:B:24:PHE:HA	0.43	2.48	7	1
1:A:13:LEU:HD11	2:B:1:PHE:CD1	0.43	2.48	7	1
2:B:3:ASN:N	2:B:3:ASN:HD22	0.42	2.12	8	1
1:A:1:GLY:HA3	1:A:19:TYR:CD2	0.42	2.49	8	1
1:A:19:TYR:N	1:A:19:TYR:CD2	0.42	2.88	4	1

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
2:B:25:PHE:CE1	2:B:25:PHE:HA	0.42	2.48	7	1
1:A:20:CYS:SG	2:B:24:PHE:CG	0.42	3.13	8	1
2:B:24:PHE:CZ	2:B:24:PHE:HB2	0.42	2.46	9	2
2:B:12:VAL:C	2:B:15:LEU:H	0.41	2.19	7	1
1:A:20:CYS:SG	2:B:24:PHE:CD1	0.40	3.14	9	2
2:B:15:LEU:HD13	2:B:15:LEU:C	0.40	2.36	4	1
2:B:11:LEU:N	2:B:11:LEU:HD13	0.40	2.31	8	1
2:B:23:GLY:HA3	2:B:24:PHE:CD1	0.40	2.51	9	2
1:A:1:GLY:HA2	1:A:19:TYR:CZ	0.40	2.52	5	1
1:A:4:GLU:CG	1:A:5:GLN:H	0.40	2.29	7	1
2:B:19:CYS:HB3	2:B:24:PHE:CD1	0.40	2.52	7	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%)	13±2 (68±9%)	4±2 (21±8%)	2±1 (12±4%)	1	7
2	B	26/30 (87%)	19±2 (71±7%)	6±2 (21±7%)	2±1 (8±5%)	2	15
All	All	450/510 (88%)	314 (70%)	94 (21%)	42 (9%)	1	11

All 14 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	22	ARG	9
1	A	5	GLN	7
2	B	26	TYR	4
1	A	9	SER	4
1	A	8	ALA	4
2	B	27	THR	2
1	A	18	ASN	2
1	A	2	ILE	2
1	A	13	LEU	2
2	B	24	PHE	2

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Mol	Chain	Res	Type	Models (Total)
1	A	10	VAL	1
2	B	3	ASN	1
2	B	16	TYR	1
2	B	21	GLU	1

6.3.2 Protein sidechains

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	19/19 (100%)	15±2 (81±9%)	4±2 (19±9%)	4 35
2	B	23/25 (92%)	16±2 (70±11%)	7±2 (30±11%)	1 16
All	All	420/440 (95%)	313 (75%)	107 (25%)	2 24

All 24 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	15	LEU	10
2	B	11	LEU	10
2	B	6	LEU	10
1	A	18	ASN	8
2	B	5	HIS	7
2	B	16	TYR	7
1	A	12	SER	7
2	B	26	TYR	6
2	B	10	HIS	6
1	A	21	ASN	5
1	A	13	LEU	5
2	B	4	GLN	4
2	B	12	VAL	4
1	A	2	ILE	3
1	A	10	VAL	3
1	A	5	GLN	2
2	B	1	PHE	2
1	A	7	CYS	2
2	B	19	CYS	1
2	B	13	GLU	1

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Mol	Chain	Res	Type	Models (Total)
1	A	11	CYS	1
2	B	18	VAL	1
1	A	20	CYS	1
2	B	3	ASN	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 monosaccharides in this entry.

6.6 Ligand geometry

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 45% for the well-defined parts and 42% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *38h_NMR_Star.txt*

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	308
Number of shifts mapped to atoms	308
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

No chemical shift referencing corrections were calculated (not enough 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 45%, i.e. 254 atoms were assigned a chemical shift out of a possible 565. 0 out of 11 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	94/240 (39%)	94/96 (98%)	0/96 (0%)	0/48 (0%)
Sidechain	130/252 (52%)	130/147 (88%)	0/96 (0%)	0/9 (0%)
Aromatic	30/73 (41%)	30/39 (77%)	0/32 (0%)	0/2 (0%)
Overall	254/565 (45%)	254/282 (90%)	0/224 (0%)	0/59 (0%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 42%, i.e. 254 atoms were assigned a chemical shift out of a possible 602. 0 out of 11 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	94/253 (37%)	94/101 (93%)	0/102 (0%)	0/50 (0%)
Sidechain	130/276 (47%)	130/162 (80%)	0/104 (0%)	0/10 (0%)
Aromatic	30/73 (41%)	30/39 (77%)	0/32 (0%)	0/2 (0%)
Overall	254/602 (42%)	254/302 (84%)	0/238 (0%)	0/62 (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: