



Full wwPDB NMR Structure Validation Report ⓘ

Apr 27, 2016 – 03:23 AM BST

PDB ID : 2MPF
Title : Solution structure human HCN2 CNBD in the cAMP-unbound state
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Deposited on : 2014-05-16

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 ⓘ symbol.

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

Cyrange : Kirchner and Güntert (2011)
NmrClust : Kelley et al. (1996)
MolProbity : 4.02b-467
Mogul : unknown
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

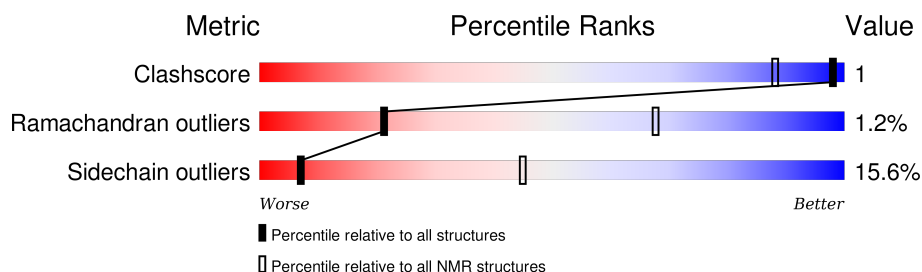
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

SOLUTION NMR

The overall completeness of chemical shifts assignment is 86%.

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 $\leq 5\%$

Mol	Chain	Length	Quality of chain
1	A	158	

2 Ensemble composition and analysis

This entry contains 20 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:535-A:655 (121)	0.77	5

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

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

The models can be grouped into 3 clusters and 2 single-model clusters were found.

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

3 Entry composition

There is only 1 type of molecule in this entry. The entry contains 2252 atoms, of which 1139 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2.

Mol	Chain	Residues	Atoms						Trace
1	A	139	Total	C	H	N	O	S	0
			2252	709	1139	195	200	9	

There are 6 discrepancies between the modelled and reference sequences:

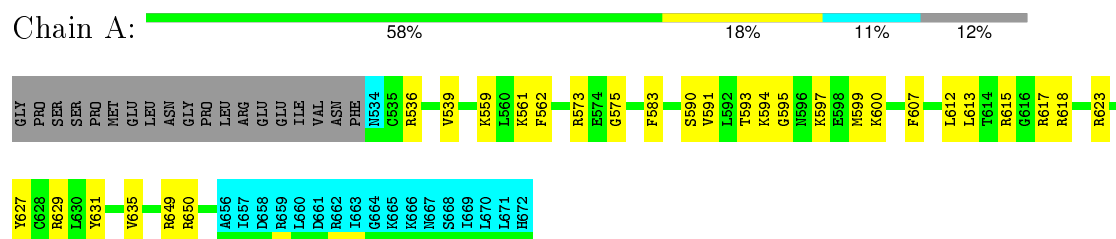
Chain	Residue	Modelled	Actual	Comment	Reference
A	515	GLY	-	EXPRESSION TAG	UNP Q9UL51
A	516	PRO	-	EXPRESSION TAG	UNP Q9UL51
A	517	SER	-	EXPRESSION TAG	UNP Q9UL51
A	518	SER	-	EXPRESSION TAG	UNP Q9UL51
A	519	PRO	-	EXPRESSION TAG	UNP Q9UL51
A	520	MET	-	EXPRESSION TAG	UNP Q9UL51

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: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2

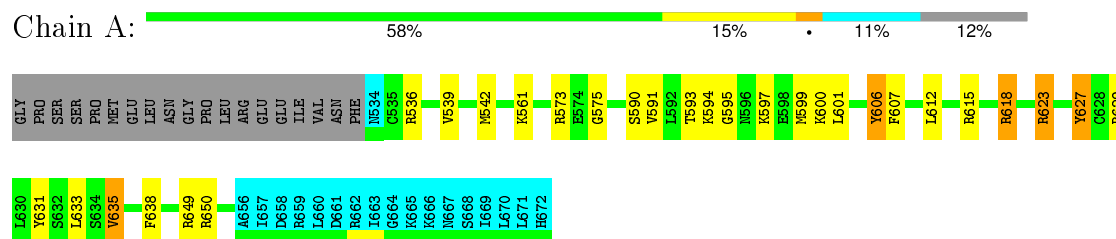


4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

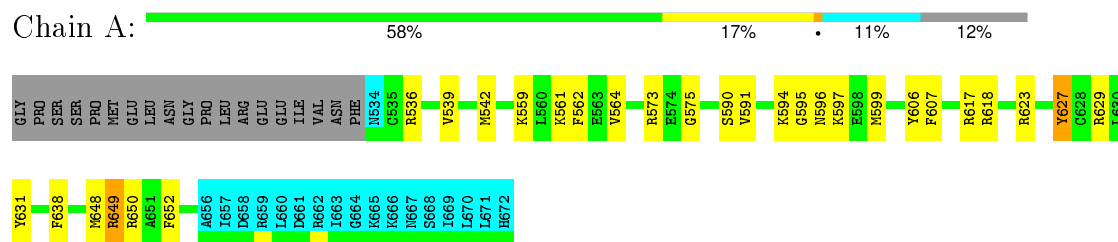
4.2.1 Score per residue for model 1

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



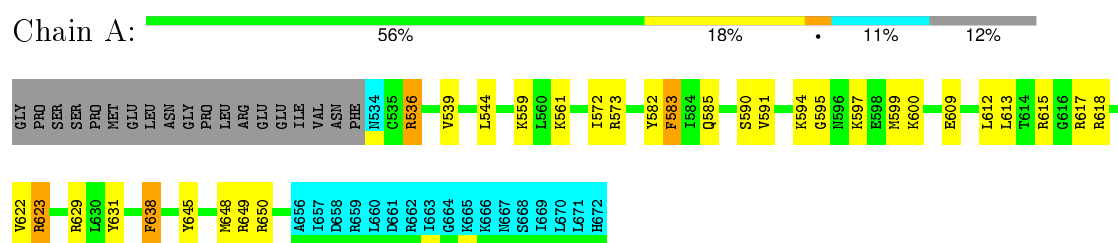
4.2.2 Score per residue for model 2

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



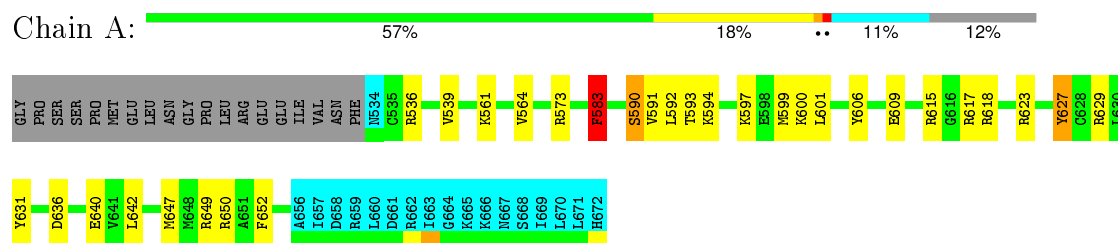
4.2.3 Score per residue for model 3

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



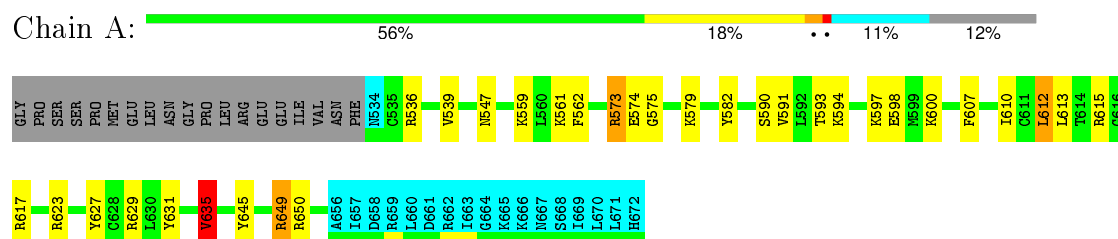
4.2.4 Score per residue for model 4

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



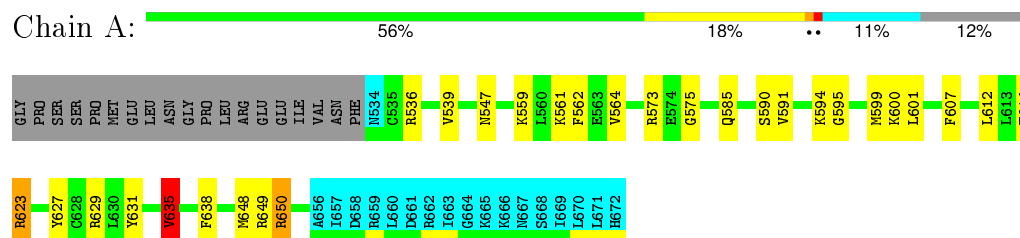
4.2.5 Score per residue for model 5 (medoid)

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



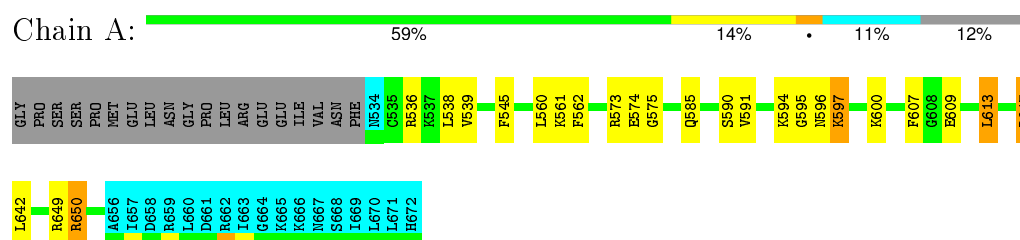
4.2.6 Score per residue for model 6

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



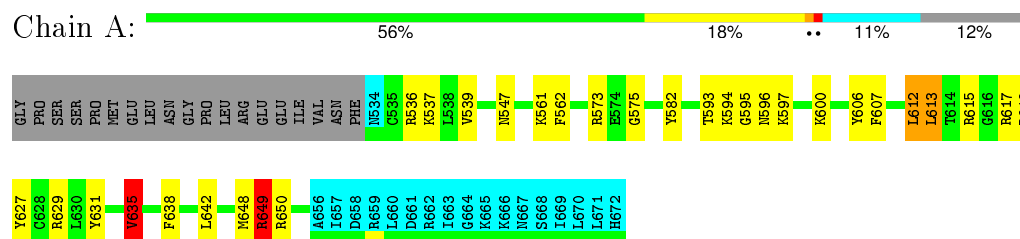
4.2.7 Score per residue for model 7

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



4.2.8 Score per residue for model 8

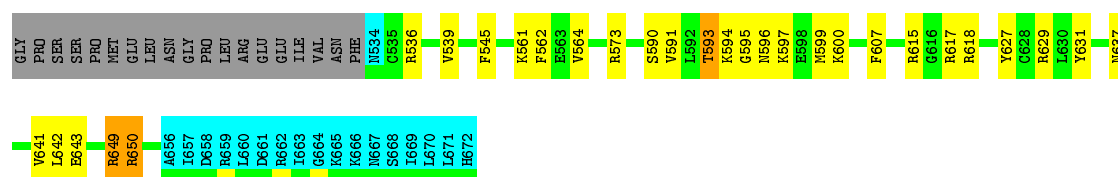
- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



4.2.9 Score per residue for model 9

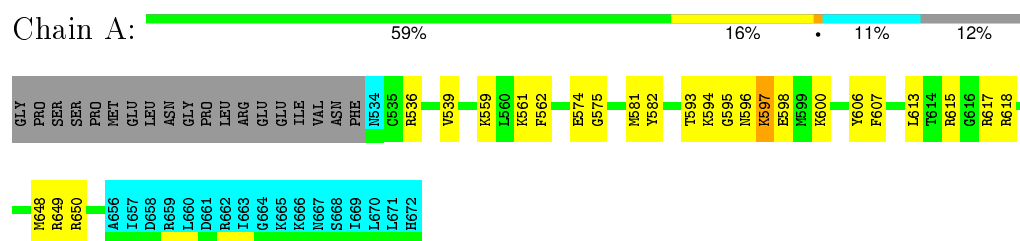
- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2





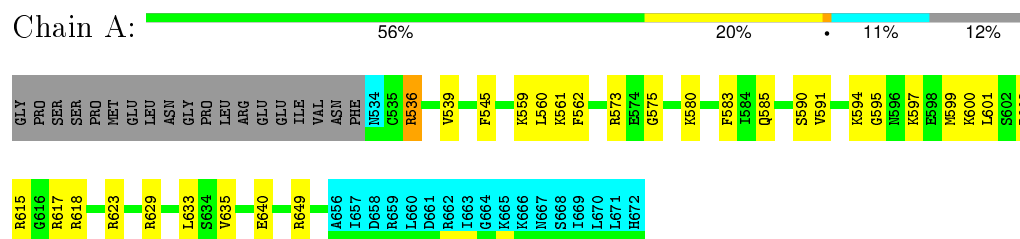
4.2.10 Score per residue for model 10

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



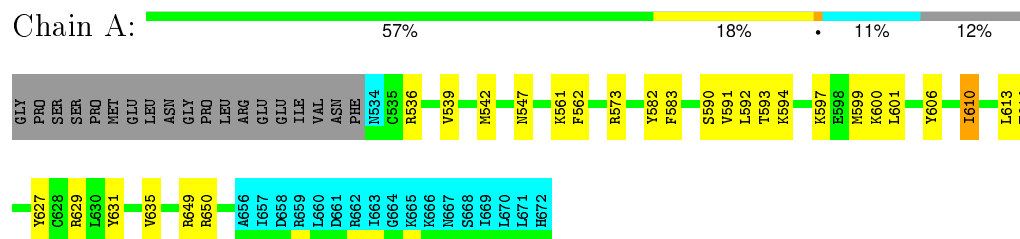
4.2.11 Score per residue for model 11

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



4.2.12 Score per residue for model 12

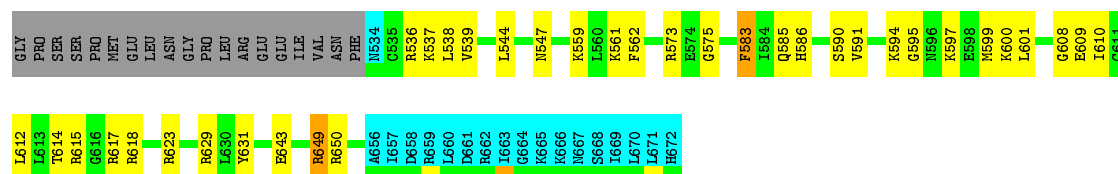
- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



4.2.13 Score per residue for model 13

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2

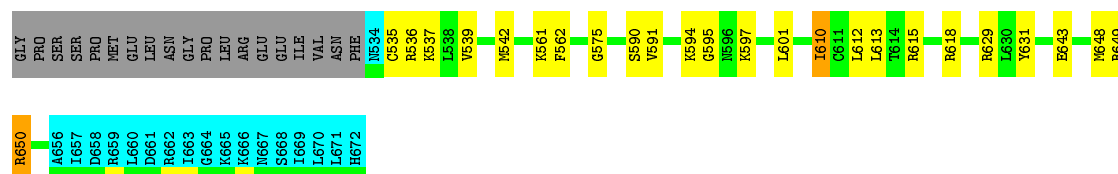
Chain A: 54% 22% 11% 12%



4.2.14 Score per residue for model 14

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2

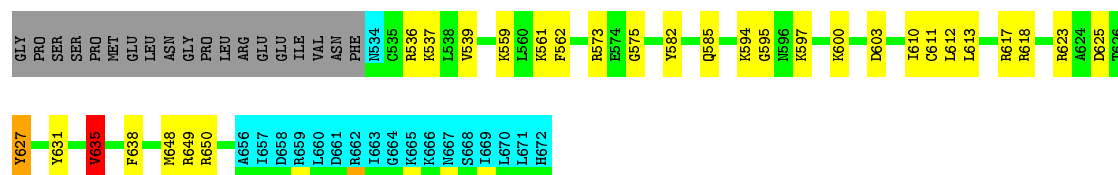
Chain A: 61% 15% 11% 12%



4.2.15 Score per residue for model 15

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2

Chain A: 58% 18% 11% 12%



4.2.16 Score per residue for model 16

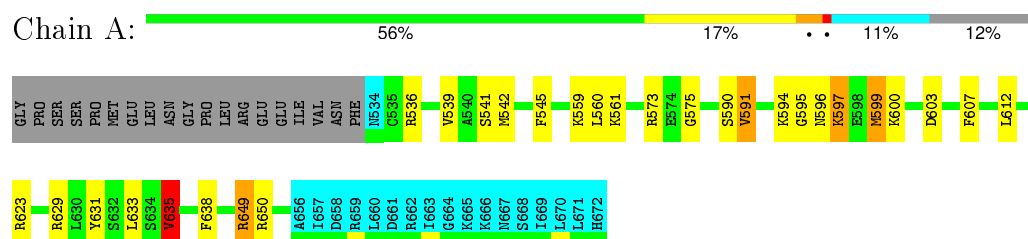
- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2

Chain A: 61% 14% 11% 12%



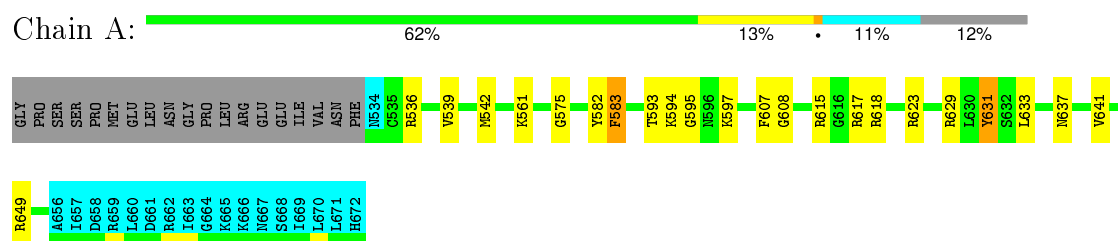
4.2.17 Score per residue for model 17

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



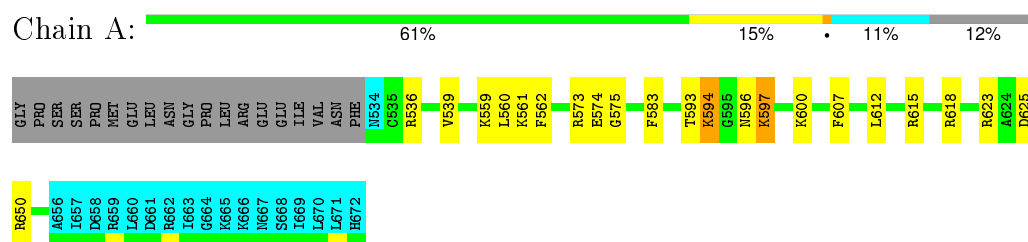
4.2.18 Score per residue for model 18

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



4.2.19 Score per residue for model 19

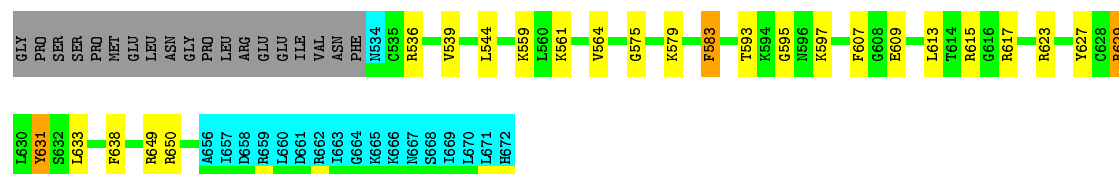
- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2



4.2.20 Score per residue for model 20

- Molecule 1: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2

Chain A: 



5 Refinement protocol and experimental data overview

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

Of the 500 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	structure solution	2.1
AMBER	refinement	12
CANDID	structure solution	
CYANA	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)	2mpf_cs.str
Number of chemical shift lists	1
Total number of shifts	1886
Number of shifts mapped to atoms	1886
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	86%

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	0.75±0.01	0±0/987 (0.0±0.0%)	1.24±0.04	10±2/1328 (0.7±0.1%)
All	All	0.75	0/19740 (0.0%)	1.24	194/26560 (0.7%)

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	3.5±1.6
All	All	0	69

There are no bond-length outliers.

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(°)	Ideal(°)	Models	
								Worst	Total
1	A	617	ARG	NE-CZ-NH1	10.89	125.75	120.30	3	16
1	A	536	ARG	NE-CZ-NH1	9.94	125.27	120.30	9	20
1	A	573	ARG	NE-CZ-NH1	9.12	124.86	120.30	15	13
1	A	649	ARG	NE-CZ-NH1	9.08	124.84	120.30	5	17
1	A	618	ARG	NE-CZ-NH1	9.02	124.81	120.30	4	17
1	A	623	ARG	NE-CZ-NH1	8.93	124.76	120.30	1	17
1	A	629	ARG	NE-CZ-NH1	8.85	124.72	120.30	1	18
1	A	615	ARG	NE-CZ-NH1	8.78	124.69	120.30	6	15
1	A	650	ARG	NE-CZ-NH1	8.68	124.64	120.30	15	16
1	A	591	VAL	CG1-CB-CG2	-7.95	98.19	110.90	17	14
1	A	583	PHE	CB-CG-CD2	-7.89	115.27	120.80	18	5
1	A	627	TYR	CB-CG-CD2	-7.78	116.33	121.00	15	2
1	A	635	VAL	CG1-CB-CG2	-7.74	98.51	110.90	5	6
1	A	583	PHE	CB-CG-CD1	7.46	126.02	120.80	18	3
1	A	631	TYR	CB-CG-CD2	-6.48	117.11	121.00	20	2

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Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)	Models	
								Worst	Total
1	A	606	TYR	CB-CG-CD2	-6.43	117.14	121.00	12	1
1	A	631	TYR	CB-CG-CD1	5.79	124.48	121.00	20	1
1	A	652	PHE	CB-CG-CD2	-5.79	116.75	120.80	2	1
1	A	622	VAL	CA-CB-CG1	5.58	119.27	110.90	3	2
1	A	627	TYR	CB-CG-CD1	5.51	124.31	121.00	15	1
1	A	638	PHE	CB-CG-CD2	-5.37	117.04	120.80	3	1
1	A	629	ARG	NE-CZ-NH2	-5.30	117.65	120.30	4	2
1	A	536	ARG	NE-CZ-NH2	-5.20	117.70	120.30	17	1
1	A	618	ARG	NE-CZ-NH2	-5.18	117.71	120.30	2	1
1	A	647	MET	CG-SD-CE	-5.14	91.98	100.20	4	1
1	A	617	ARG	NE-CZ-NH2	-5.09	117.75	120.30	3	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)
1	A	595	GLY	Peptide	16
1	A	590	SER	Peptide	14
1	A	599	MET	Peptide	11
1	A	545	PHE	Sidechain	4
1	A	606	TYR	Sidechain	4
1	A	597	LYS	Peptide	2
1	A	610	ILE	Peptide	2
1	A	649	ARG	Sidechain	2
1	A	645	TYR	Sidechain	2
1	A	607	PHE	Peptide	2
1	A	582	TYR	Sidechain	2
1	A	593	THR	Peptide	1
1	A	631	TYR	Sidechain	1
1	A	613	LEU	Peptide	1
1	A	608	GLY	Peptide	1
1	A	592	LEU	Peptide	1
1	A	596	ASN	Peptide	1
1	A	629	ARG	Sidechain	1
1	A	572	ILE	Peptide	1

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	968	981	981	1±1
All	All	19360	19620	19620	22

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 1.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:612:LEU:HD23	1:A:635:VAL:HG22	0.62	1.71	17	6
1:A:637:ASN:O	1:A:641:VAL:HG12	0.57	2.00	18	2
1:A:564:VAL:HG11	1:A:627:TYR:CE1	0.48	2.42	9	5
1:A:613:LEU:H	1:A:613:LEU:HD13	0.45	1.72	8	1
1:A:596:ASN:CA	1:A:597:LYS:HE3	0.45	2.42	7	3
1:A:596:ASN:HA	1:A:597:LYS:HE3	0.43	1.88	10	1
1:A:591:VAL:HG12	1:A:599:MET:C	0.43	2.33	17	1
1:A:591:VAL:HG12	1:A:599:MET:O	0.43	2.14	17	1
1:A:583:PHE:CE1	1:A:631:TYR:CE2	0.41	3.08	4	1
1:A:560:LEU:HD23	1:A:561:LYS:N	0.41	2.30	17	1

6.3 Torsion angles

6.3.1 Protein backbone

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	121/158 (77%)	115±1 (95±1%)	4±1 (3±1%)	2±1 (1±1%)	21	68
All	All	2420/3160 (77%)	2307 (95%)	83 (3%)	30 (1%)	21	68

All 8 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	A	575	GLY	15
1	A	610	ILE	5
1	A	612	LEU	4
1	A	609	GLU	2
1	A	594	LYS	1
1	A	608	GLY	1
1	A	611	CYS	1
1	A	535	CYS	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	106/139 (76%)	90±3 (84±3%)	17±3 (16±3%)	7	45
All	All	2120/2780 (76%)	1790 (84%)	330 (16%)	7	45

All 57 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	539	VAL	20
1	A	594	LYS	19
1	A	597	LYS	19
1	A	561	LYS	19
1	A	600	LYS	16
1	A	631	TYR	15
1	A	562	PHE	14
1	A	559	LYS	12
1	A	607	PHE	11
1	A	613	LEU	10
1	A	593	THR	10
1	A	638	PHE	9
1	A	583	PHE	8
1	A	648	MET	8
1	A	623	ARG	7
1	A	627	TYR	7
1	A	635	VAL	7
1	A	601	LEU	7

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Mol	Chain	Res	Type	Models (Total)
1	A	649	ARG	7
1	A	585	GLN	7
1	A	542	MET	6
1	A	582	TYR	5
1	A	625	ASP	5
1	A	547	ASN	5
1	A	650	ARG	5
1	A	633	LEU	5
1	A	573	ARG	4
1	A	537	LYS	4
1	A	642	LEU	4
1	A	574	GLU	4
1	A	614	THR	3
1	A	609	GLU	3
1	A	538	LEU	3
1	A	560	LEU	3
1	A	596	ASN	3
1	A	603	ASP	3
1	A	544	LEU	3
1	A	643	GLU	3
1	A	606	TYR	2
1	A	640	GLU	2
1	A	536	ARG	2
1	A	598	GLU	2
1	A	612	LEU	2
1	A	592	LEU	2
1	A	579	LYS	2
1	A	629	ARG	2
1	A	586	HIS	1
1	A	581	MET	1
1	A	636	ASP	1
1	A	615	ARG	1
1	A	634	SER	1
1	A	590	SER	1
1	A	617	ARG	1
1	A	652	PHE	1
1	A	580	LYS	1
1	A	541	SER	1
1	A	618	ARG	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 carbohydrates 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 86% for the well-defined parts and 83% for the entire structure.

7.1 Chemical shift list 1

File name: 2mpf_cs.str

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	1886
Number of shifts mapped to atoms	1886
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	2

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction \pm precision, ppm	Suggested action
$^{13}\text{C}_\alpha$	152	0.07 ± 0.23	None needed (< 0.5 ppm)
$^{13}\text{C}_\beta$	142	0.12 ± 0.15	None needed (< 0.5 ppm)
$^{13}\text{C}'$	131	0.14 ± 0.18	None needed (< 0.5 ppm)
^{15}N	139	0.51 ± 0.46	None needed (imprecise)

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 86%, i.e. 1312 atoms were assigned a chemical shift out of a possible 1530. 18 out of 21 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^1H	^{13}C	^{15}N
Backbone	568/597 (95%)	232/238 (97%)	224/242 (93%)	112/117 (96%)
Sidechain	681/806 (84%)	419/474 (88%)	254/290 (88%)	8/42 (19%)

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	Total	¹H	¹³C	¹⁵N
Aromatic	63/127 (50%)	63/68 (93%)	0/58 (0%)	0/1 (0%)
Overall	1312/1530 (86%)	714/780 (92%)	478/590 (81%)	120/160 (75%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 83%, i.e. 1479 atoms were assigned a chemical shift out of a possible 1773. 21 out of 24 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	¹H	¹³C	¹⁵N
Backbone	636/687 (93%)	260/274 (95%)	251/278 (90%)	125/135 (93%)
Sidechain	779/952 (82%)	478/559 (86%)	293/341 (86%)	8/52 (15%)
Aromatic	64/134 (48%)	64/72 (89%)	0/60 (0%)	0/2 (0%)
Overall	1479/1773 (83%)	802/905 (89%)	544/679 (80%)	133/189 (70%)

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	582	TYR	HB3	0.61	4.75 – 0.95	-5.9
1	A	571	ILE	HG13	-1.10	3.26 – -0.84	-5.6

7.1.5 Random Coil Index (RCI) plots ⓘ

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:

