



Full wwPDB NMR Structure Validation Report ⓘ

Feb 13, 2017 – 12:40 am GMT

PDB ID : 2M3O
Title : Structure and dynamics of a human Nedd4 WW domain-ENaC complex
Authors : Bobby, R.; Medini, K.; Neudecker, P.; Lee, V.; MacDonald, F.J.; Brimble, M.A.; Lott, J.; Dingley, A.J.
Deposited on : 2013-01-23

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
Percentile statistics : 20161228.v01 (using entries in the PDB archive December 28th 2016)
RCI : v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV : Wang et al. (2010)
ShiftChecker : trunk28760
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : recalc28949

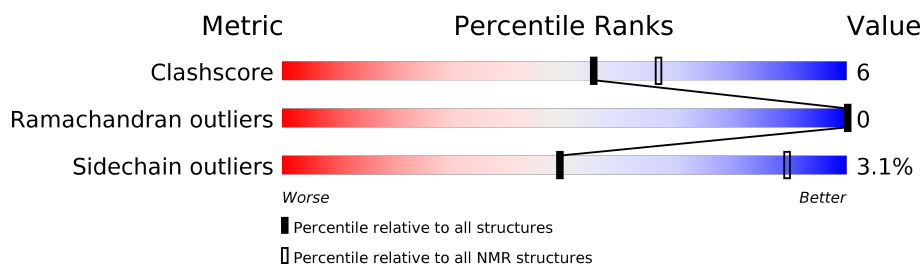
1 Overall quality at a glance

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	125131	11601
Ramachandran outliers	121729	10391
Sidechain outliers	121581	10367

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	W	43	
2	P	11	

2 Ensemble composition and analysis

This entry contains 15 models. Model 6 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	W:423-W:455, P:640-P:648 (42)	0.48	6

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 1 single-model cluster was found.

Cluster number	Models
1	1, 4, 6, 7, 8, 9, 11, 12
2	10, 13, 15
3	2, 3, 5
Single-model clusters	14

3 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 839 atoms, of which 414 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called E3 ubiquitin-protein ligase NEDD4.

Mol	Chain	Residues	Atoms						Trace
1	W	43	Total	C	H	N	O	S	0
			692	225	341	64	61	1	

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
W	416	GLY	-	EXPRESSION TAG	UNP P46934
W	417	SER	-	EXPRESSION TAG	UNP P46934
W	418	MET	-	EXPRESSION TAG	UNP P46934

- Molecule 2 is a protein called Amiloride-sensitive sodium channel subunit alpha.

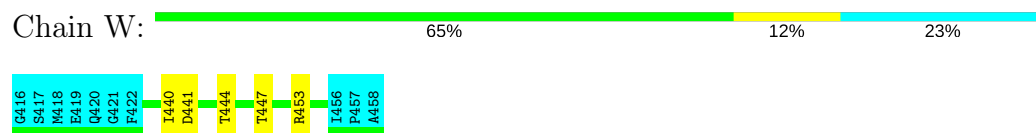
Mol	Chain	Residues	Atoms					Trace
2	P	11	Total	C	H	N	O	0
			147	49	73	11	14	

4 Residue-property plots

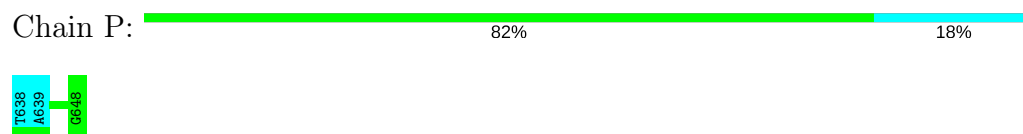
4.1 Average score per residue in the NMR ensemble

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: E3 ubiquitin-protein ligase NEDD4



- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

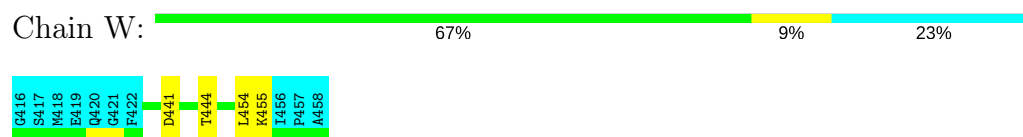


4.2 Scores per residue for each member of the ensemble

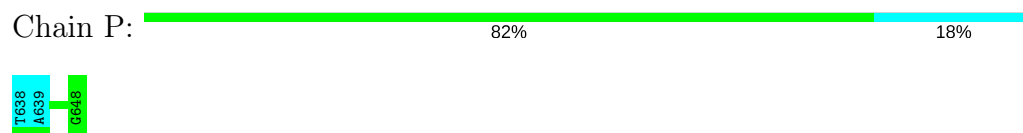
Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

- Molecule 1: E3 ubiquitin-protein ligase NEDD4



- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

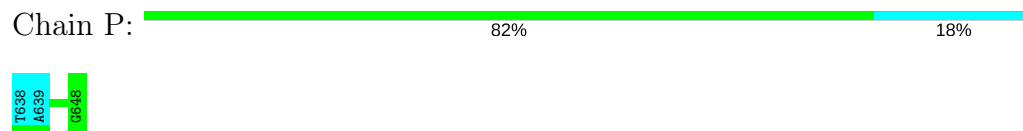


4.2.2 Score per residue for model 2

- Molecule 1: E3 ubiquitin-protein ligase NEDD4



- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

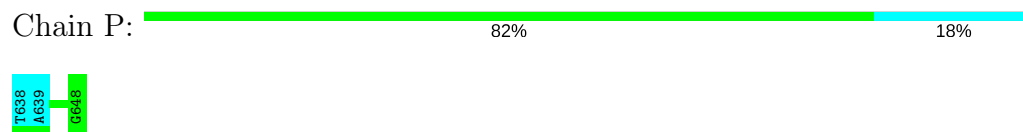


4.2.3 Score per residue for model 3

- Molecule 1: E3 ubiquitin-protein ligase NEDD4



- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

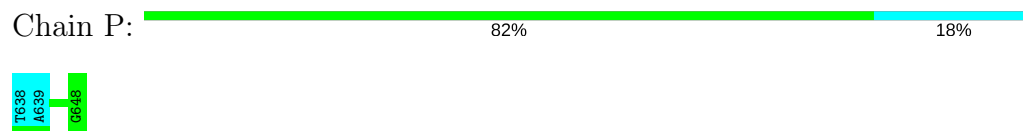


4.2.4 Score per residue for model 4

- Molecule 1: E3 ubiquitin-protein ligase NEDD4

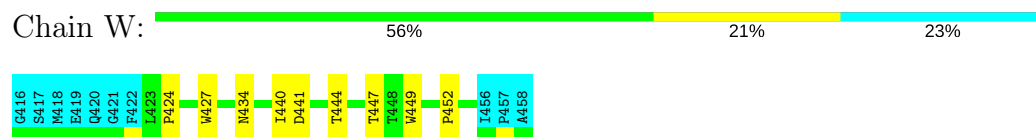


- Molecule 2: Amiloride-sensitive sodium channel subunit alpha



4.2.5 Score per residue for model 5

- Molecule 1: E3 ubiquitin-protein ligase NEDD4



- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

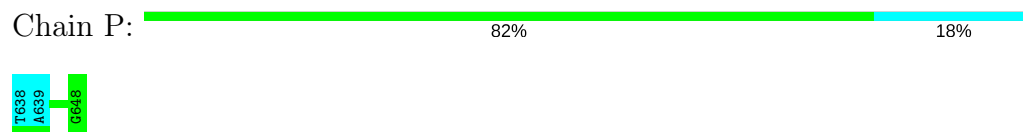


4.2.6 Score per residue for model 6 (medoid)

- Molecule 1: E3 ubiquitin-protein ligase NEDD4

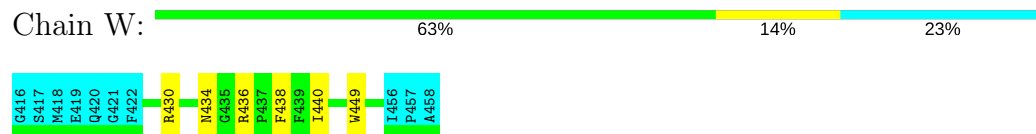


- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

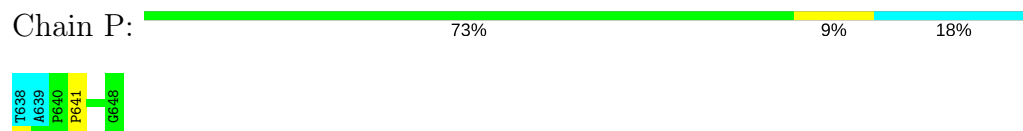


4.2.7 Score per residue for model 7

- Molecule 1: E3 ubiquitin-protein ligase NEDD4

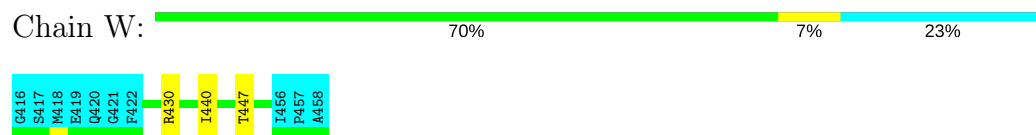


- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

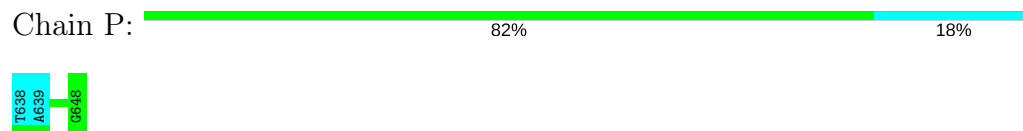


4.2.8 Score per residue for model 8

- Molecule 1: E3 ubiquitin-protein ligase NEDD4



- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

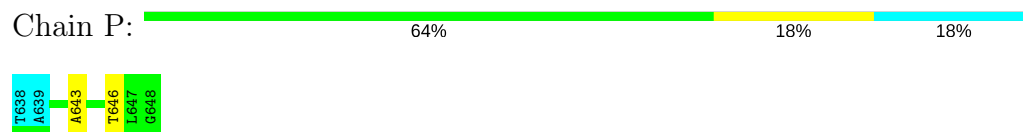


4.2.9 Score per residue for model 9

- Molecule 1: E3 ubiquitin-protein ligase NEDD4

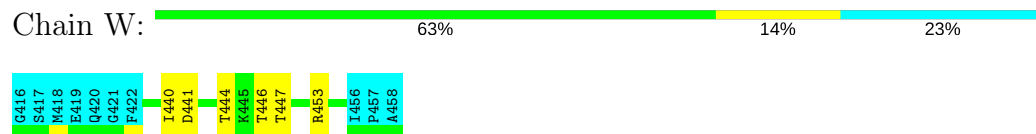


- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

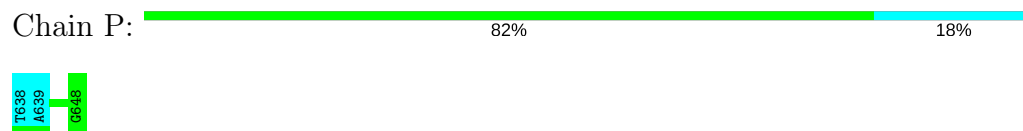


4.2.10 Score per residue for model 10

- Molecule 1: E3 ubiquitin-protein ligase NEDD4



- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

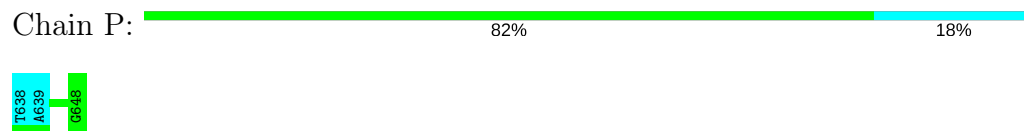


4.2.11 Score per residue for model 11

- Molecule 1: E3 ubiquitin-protein ligase NEDD4



- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

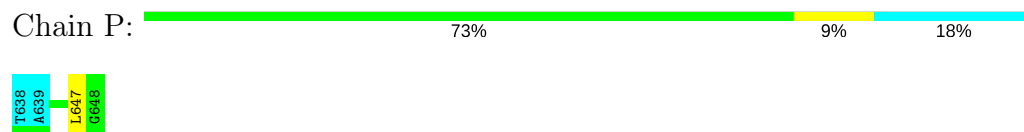


4.2.12 Score per residue for model 12

- Molecule 1: E3 ubiquitin-protein ligase NEDD4

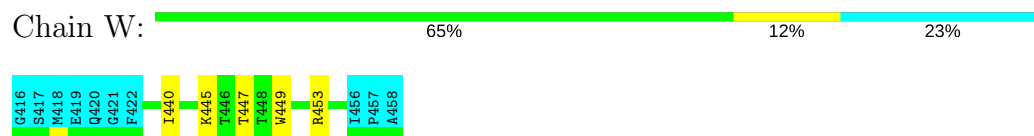


- Molecule 2: Amiloride-sensitive sodium channel subunit alpha



4.2.13 Score per residue for model 13

- Molecule 1: E3 ubiquitin-protein ligase NEDD4



- Molecule 2: Amiloride-sensitive sodium channel subunit alpha



4.2.14 Score per residue for model 14

- Molecule 1: E3 ubiquitin-protein ligase NEDD4

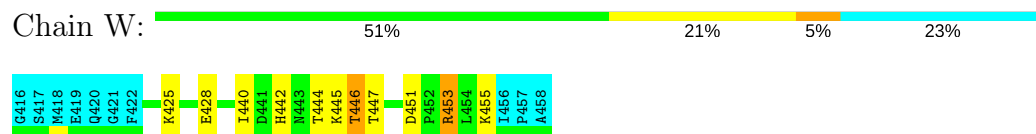


- Molecule 2: Amiloride-sensitive sodium channel subunit alpha

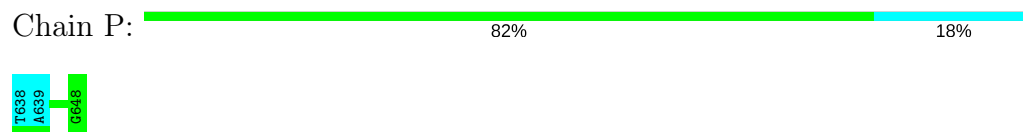


4.2.15 Score per residue for model 15

- Molecule 1: E3 ubiquitin-protein ligase NEDD4



- Molecule 2: Amiloride-sensitive sodium channel subunit alpha



5 Refinement protocol and experimental data overview

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

Of the 200 calculated structures, 15 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
CYANA	structure solution	2.1
CNS	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)	2m3o_cs.str
Number of chemical shift lists	2
Total number of shifts	640
Number of shifts mapped to atoms	640
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

There are no covalent bond-length or bond-angle outliers.

There are no bond-length outliers.

There are no bond-angle outliers.

There are no chirality outliers.

There are no planarity outliers.

6.2 Too-close contacts

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in each chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes averaged over the ensemble.

Mol	Chain	Non-H	H(model)	H(added)	Clashes
1	W	280	273	271	4±2
2	P	62	61	61	1±1
All	All	5130	5010	4980	62

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 6.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:W:454:LEU:HD12	1:W:455:LYS:HG3	0.71	1.60	1	1
1:W:453:ARG:HE	1:W:453:ARG:HA	0.71	1.44	3	1
1:W:453:ARG:HA	1:W:453:ARG:NE	0.70	2.01	11	4
1:W:453:ARG:NE	1:W:453:ARG:HA	0.67	2.04	3	2
1:W:440:ILE:HG12	1:W:447:THR:HG22	0.65	1.67	9	8
1:W:453:ARG:HA	1:W:453:ARG:HE	0.60	1.57	14	3
1:W:440:ILE:HD13	2:P:647:LEU:HD13	0.58	1.75	12	1
1:W:444:THR:HG23	1:W:446:THR:HG22	0.56	1.75	10	1
1:W:424:PRO:HG2	1:W:427:TRP:CD1	0.56	2.35	5	1
1:W:423:LEU:HB3	1:W:427:TRP:HB2	0.55	1.79	12	1
2:P:642:PRO:HG2	2:P:647:LEU:HD21	0.53	1.80	5	1
1:W:449:TRP:HZ2	2:P:641:PRO:HD3	0.53	1.64	7	1
1:W:448:THR:OG1	1:W:450:GLU:HG2	0.52	2.05	4	3

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:W:444:THR:HG23	1:W:446:THR:HB	0.52	1.81	15	1
1:W:441:ASP:OD2	1:W:444:THR:HG22	0.50	2.06	10	8
1:W:449:TRP:CZ2	2:P:641:PRO:HD3	0.48	2.43	5	3
1:W:451:ASP:O	1:W:455:LYS:HG2	0.47	2.09	11	2
1:W:438:PHE:CE1	1:W:440:ILE:HD11	0.46	2.46	7	1
1:W:452:PRO:HG2	1:W:453:ARG:HD3	0.46	1.88	4	1
1:W:430:ARG:HB3	1:W:438:PHE:CE1	0.46	2.45	6	1
1:W:428:GLU:HB3	1:W:440:ILE:HB	0.45	1.88	9	1
1:W:424:PRO:HB2	1:W:427:TRP:CD1	0.45	2.46	3	2
2:P:643:ALA:O	2:P:647:LEU:HG	0.45	2.11	14	1
2:P:643:ALA:O	2:P:646:THR:HG22	0.44	2.12	9	1
1:W:440:ILE:HG12	1:W:447:THR:HG23	0.44	1.88	6	1
1:W:428:GLU:HB2	1:W:442:HIS:NE2	0.44	2.28	9	2
1:W:440:ILE:N	1:W:440:ILE:HD12	0.43	2.28	7	1
1:W:453:ARG:CA	1:W:453:ARG:HE	0.43	2.23	3	2
2:P:644:TYR:HA	2:P:647:LEU:HG	0.43	1.91	13	1
1:W:427:TRP:HZ3	1:W:448:THR:HG21	0.42	1.73	14	1
1:W:444:THR:CG2	1:W:446:THR:HG22	0.42	2.43	10	1
1:W:451:ASP:OD2	1:W:453:ARG:HB2	0.42	2.15	15	1
1:W:427:TRP:HA	1:W:440:ILE:O	0.42	2.14	11	1
1:W:453:ARG:CA	1:W:453:ARG:NE	0.40	2.81	12	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	W	33/43 (77%)	31±1 (93±4%)	2±1 (7±4%)	0±0 (0±0%)	100	100
2	P	8/11 (73%)	8±0 (99±3%)	0±0 (1±3%)	0±0 (0±0%)	100	100
All	All	615/810 (76%)	579 (94%)	36 (6%)	0 (0%)	100	100

There are no Ramachandran outliers.

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	W	30/37 (81%)	29±1 (96±4%)	1±1 (4±4%)	42	86
2	P	6/7 (86%)	6±0 (100±0%)	0±0 (0±0%)	100	100
All	All	540/660 (82%)	523 (97%)	17 (3%)	49	89

All 8 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	W	430	ARG	5
1	W	455	LYS	3
1	W	445	LYS	2
1	W	425	LYS	2
1	W	453	ARG	2
1	W	446	THR	1
1	W	436	ARG	1
1	W	434	ASN	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 [i](#)

The completeness of assignment taking into account all chemical shift lists is 86% for the well-defined parts and 85% for the entire structure.

7.1 Chemical shift list 1

File name: 2m3o_cs.str

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	520
Number of shifts mapped to atoms	520
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	3

7.1.2 Chemical shift referencing [i](#)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction \pm precision, ppm	Suggested action
$^{13}\text{C}_\alpha$	43	0.39 ± 0.19	None needed (< 0.5 ppm)
$^{13}\text{C}_\beta$	39	0.07 ± 0.20	None needed (< 0.5 ppm)
$^{13}\text{C}'$	42	0.16 ± 0.30	None needed (< 0.5 ppm)
^{15}N	42	0.46 ± 0.73	None needed (< 0.5 ppm)

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 69%, i.e. 376 atoms were assigned a chemical shift out of a possible 543. 3 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^1H	^{13}C	^{15}N
Backbone	157/196 (80%)	62/77 (81%)	66/84 (79%)	29/35 (83%)
Sidechain	201/281 (72%)	124/169 (73%)	72/98 (73%)	5/14 (36%)

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	Total	¹ H	¹³ C	¹⁵ N
Aromatic	18/66 (27%)	16/34 (47%)	0/26 (0%)	2/6 (33%)
Overall	376/543 (69%)	202/280 (72%)	138/208 (66%)	36/55 (65%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 71%, i.e. 472 atoms were assigned a chemical shift out of a possible 667. 3 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	¹ H	¹³ C	¹⁵ N
Backbone	202/254 (80%)	79/100 (79%)	85/108 (79%)	38/46 (83%)
Sidechain	248/338 (73%)	154/203 (76%)	88/120 (73%)	6/15 (40%)
Aromatic	22/75 (29%)	20/39 (51%)	0/30 (0%)	2/6 (33%)
Overall	472/667 (71%)	253/342 (74%)	173/258 (67%)	46/67 (69%)

7.1.4 Statistically unusual chemical shifts ⓘ

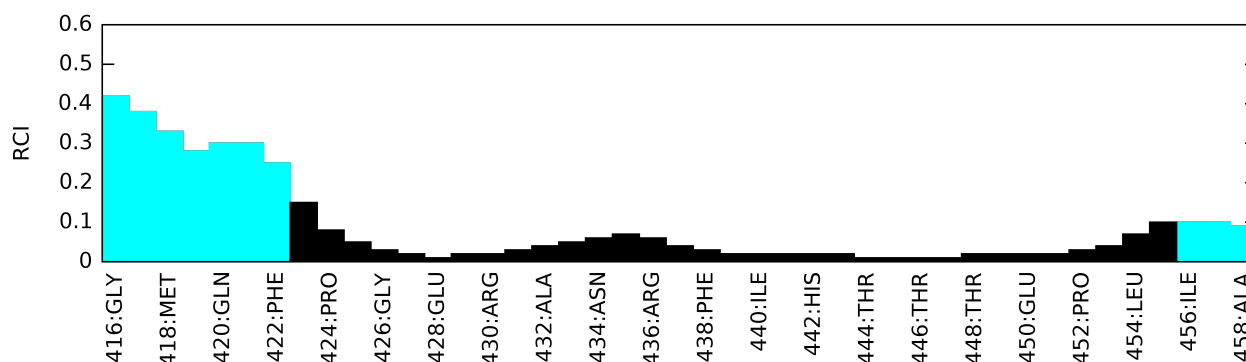
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
???	W	451	ASP	HA	2.47	6.15 – 3.05	-6.9
???	W	452	PRO	HG3	0.21	3.56 – 0.26	-5.1
???	W	436	ARG	HD2	1.96	4.27 – 1.97	-5.0

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 W:



7.2 Chemical shift list 2

File name: 2m3o_cs.str

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	120
Number of shifts mapped to atoms	120
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

7.2.2 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 16%, i.e. 89 atoms were assigned a chemical shift out of a possible 543. 1 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	¹ H	¹³ C	¹⁵ N
Backbone	36/196 (18%)	15/77 (19%)	15/84 (18%)	6/35 (17%)
Sidechain	45/281 (16%)	28/169 (17%)	17/98 (17%)	0/14 (0%)
Aromatic	8/66 (12%)	4/34 (12%)	4/26 (15%)	0/6 (0%)

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	Total	¹ H	¹³ C	¹⁵ N
Overall	89/543 (16%)	47/280 (17%)	36/208 (17%)	6/55 (11%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 15%, i.e. 98 atoms were assigned a chemical shift out of a possible 667. 1 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	¹ H	¹³ C	¹⁵ N
Backbone	41/254 (16%)	17/100 (17%)	17/108 (16%)	7/46 (15%)
Sidechain	49/338 (14%)	30/203 (15%)	19/120 (16%)	0/15 (0%)
Aromatic	8/75 (11%)	4/39 (10%)	4/30 (13%)	0/6 (0%)
Overall	98/667 (15%)	51/342 (15%)	40/258 (16%)	7/67 (10%)

7.2.4 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
???	P	642	PRO	HD3	1.11	5.52 – 1.72	-6.6
???	P	641	PRO	HB2	-0.17	3.82 – 0.32	-6.4

7.2.5 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 P:

