

Full wwPDB NMR Structure Validation Report (i)

Apr 20, 2024 – 11:46 PM EDT

PDB ID : 2M3O BMRB ID : 18971

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,

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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
https://www.wwpdb.org/validation/2017/NMRValidationReportHelp
with specific help available everywhere you see the (i) symbol.

The types of validation reports are described at http://www.wwpdb.org/validation/2017/FAQs#types.

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

MolProbity: 4.02b-467

Percentile statistics : 20191225.v01 (using entries in the PDB archive December 25th 2019)

wwPDB-RCI : v 1n 11 5 13 A (Berjanski et al., 2005)

PANAV : Wang et al. (2010)

wwPDB-ShiftChecker : v1.2

Ideal geometry (proteins) : Engh & Huber (2001) Ideal geometry (DNA, RNA) : Parkinson et al. (1996)

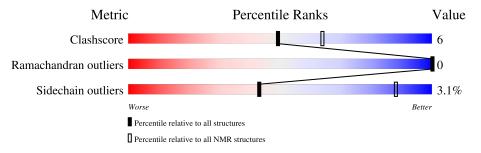
Validation Pipeline (wwPDB-VP) : 2.36.2

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 88%.

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	NMR archive
Metric	$(\# \mathrm{Entries})$	$(\# { m Entries})$
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	W	43	65%	12%	23%	
2	Р	11	82%		18%	



2 Ensemble composition and analysis (i)

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	0.84	6		
	(42)				

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 (i)

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	49	Total	С	Н	N	О	S	0
1	VV	43	692	225	341	64	61	1	U

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	
9	D	11	Total	С	Н	N	О	0
	2 P	P 11	147	49	73	11	14	U

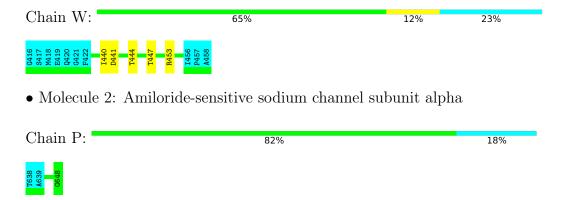


4 Residue-property plots (i)

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: E3 ubiquitin-protein ligase NEDD4



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

Chain W:	67%	9%	23%		
6416 S417 M4 18 E419 G420 G421 F422 D441	1444 1456 1466 1468 1468 1468				
• Molecule 2: Amiloride-sensitive sodium channel subunit alpha					
Chain P:	82%		18%		





4.2.2 Score per residue for model 2

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 63% 14% 23%

6416 8417 8417 6420 6421 7422 1440 1941 1744 7445 7455 1456 8455 1456

• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 82% 18%



4.2.3 Score per residue for model 3

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 63% 14% 23%

0416 8417 8417 8418 6420 6421 1423 P424 P424 P427 P424 P427 P427 P427 P441 T444 T446 P457 A458

• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 82% 18%



4.2.4 Score per residue for model 4

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 58% 16% • 23%



• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 82% 18%





4.2.5 Score per residue for model 5

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 56% 21% 23%

0416 M418 M418 E419 G421 F422 F423 M427 M427 M448 M449 P465 P466 P466

• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 55% 27% 18%

T638 A639 P640 P641 P642 L647 G648

4.2.6 Score per residue for model 6 (medoid)

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 63% 14% 23%

6416 8417 9417 9420 9421 6421 6421 6438 6438 1440 D441 1444 1444 1447 1447 1447

• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 82% 18%



4.2.7 Score per residue for model 7

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 63% 14% 23%



• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 73% 9% 18%





4.2.8 Score per residue for model 8

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 70% 7% 23%

6416 8417 8417 6421 6421 6422 6421 1440 1447 1447 1456 1456 1456

• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 82% 18%



4.2.9 Score per residue for model 9

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 63% 14% 23%

G416 M418 E419 G421 G421 G421 G421 G421 G429 B430 B442 H442 T447 T447 T447 T447 A458

• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 64% 18% 18%



4.2.10 Score per residue for model 10

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 63% 14% 23%



• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 82% 18%





4.2.11 Score per residue for model 11

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 60% 16% 23%

• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 82% 18%



4.2.12 Score per residue for model 12

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 58% 19% 23%

• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 73% 9% 18%



4.2.13 Score per residue for model 13

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 65% 12% 23%



• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 55% 27% 18%





4.2.14 Score per residue for model 14

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 63% 14% 23%

• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 64% 18% 18%



4.2.15 Score per residue for model 15

• Molecule 1: E3 ubiquitin-protein ligase NEDD4

Chain W: 51% 21% 5% 23%

• Molecule 2: Amiloride-sensitive sodium channel subunit alpha

Chain P: 82% 18%





5 Refinement protocol and experimental data overview (i)



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)	working_cs.cif
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)	88%



6 Model quality (i)

6.1 Standard geometry (i)

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

There are no bond-length outliers.

There are no bond-angle outliers.

There are no chirality outliers.

There are no planarity outliers.

6.2 Too-close contacts (i)

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

Mol	Chain	Non-H	H(model)	H(added)	Clashes
1	W	280	273	271	4 ± 2
2	Р	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)	$Distance(\mathring{A})$	Models	
Atom-1	Atom-2	Clash(A)	Distance(A)	Worst	Total
1:W:454:LEU:HD12	1:W:455:LYS:HG3	0.71	1.60	1	1
1:W:453:ARG:HA	1:W:453:ARG:HE	0.71	1.44	3	4
1:W:453:ARG:HA	1:W:453:ARG:NE	0.70	2.01	11	6
1:W:440:ILE:HG12	1:W:447:THR:HG22	0.65	1.67	9	8
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
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

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
Atom-1	Atom-2	Clash(A)	Distance(A)	Worst	Total
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:HD12	1:W:440:ILE:N	0.43	2.28	7	1
1:W:453:ARG:HE	1:W:453:ARG:CA	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:NE	1:W:453:ARG:CA	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	Percei	ntiles
1	W	33/43 (77%)	31±1 (93±4%)	2±1 (7±4%)	0±0 (0±0%)	100	100
2	Р	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	Percei	ntiles
1	W	30/37 (81%)	29±1 (96±4%)	1±1 (4±4%)	36	84
2	Р	6/7 (86%)	6±0 (100±0%)	0±0 (0±0%)	100	100
All	All	540/660 (82%)	523 (97%)	17 (3%)	43	88

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	425	LYS	2
1	W	453	ARG	2
1	W	445	LYS	2
1	W	434	ASN	1
1	W	436	ARG	1
1	W	446	THR	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 monosaccharides 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 88% for the well-defined parts and 87% for the entire structure.

7.1 Chemical shift list 1

File name: working cs.cif

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
$^{13}\mathrm{C}_{\alpha}$	43	0.39 ± 0.19	None needed ($< 0.5 \text{ ppm}$)
$^{13}C_{\beta}$	39	0.07 ± 0.25	None needed ($< 0.5 \text{ ppm}$)
¹³ C′	42	0.17 ± 0.30	None needed ($< 0.5 \text{ ppm}$)
^{15}N	42	0.40 ± 0.88	None needed ($< 0.5 \text{ ppm}$)

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 70%, i.e. 412 atoms were assigned a chemical shift out of a possible 585. 0 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathbf{H}$	$^{13}\mathbf{C}$	$^{15}\mathbf{N}$
Backbone	159/199 (80%)	64/80 (80%)	66/84~(79%)	29/35 (83%)
Sidechain	235/317 (74%)	154/205 (75%)	76/98 (78%)	5/14 (36%)

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	Total	$^{1}\mathbf{H}$	$^{13}\mathbf{C}$	$^{15}\mathbf{N}$
Aromatic	18/69 (26%)	16/34 (47%)	0/29~(0%)	2/6 (33%)
Overall	412/585 (70%)	234/319 (73%)	142/211 (67%)	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. 516 atoms were assigned a chemical shift out of a possible 724. 0 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}{ m H}$	$^{13}\mathbf{C}$	$^{15}{ m N}$
Backbone	206/259 (80%)	83/105 (79%)	85/108 (79%)	38/46 (83%)
Sidechain	288/386 (75%)	190/251 (76%)	92/120 (77%)	6/15 (40%)
Aromatic	22/79 (28%)	20/39 (51%)	0/34 (0%)	2/6 (33%)
Overall	516/724 (71%)	293/395 (74%)	177/262 (68%)	46/67 (69%)

7.1.4 Statistically unusual chemical shifts (i)

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

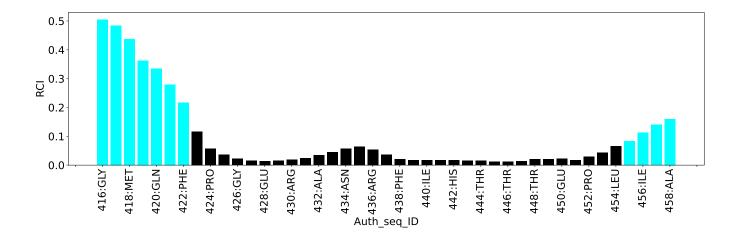
List Id	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	W	451	ASP	HA	2.47	3.04 - 6.12	-6.8
1	W	452	PRO	HG3	0.21	0.33 - 3.48	-5.4
1	W	436	ARG	HD2	1.96	1.97 - 4.26	-5.0

7.1.5 Random Coil Index (RCI) plots (i)

The image below reports random coil index values for the protein chains in the structure. The height of each bar gives a probability of a given residue to be disordered, as predicted from the available chemical shifts and the amino acid sequence. A value above 0.2 is an indication of significant predicted disorder. The colour of the bar shows whether the residue is in the well-defined core (black) or in the ill-defined residue ranges (cyan), as described in section 2 on ensemble composition. If well-defined core and ill-defined regions are not identified then it is shown as gray bars.

Random coil index (RCI) for chain W:





7.2 Chemical shift list 2

File name: working_cs.cif

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 17%, i.e. 102 atoms were assigned a chemical shift out of a possible 585. 0 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}{ m H}$	$^{13}\mathbf{C}$	$^{15}{ m N}$
Backbone	37/199 (19%)	16/80~(20%)	15/84~(18%)	6/35 (17%)

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	Total	$^{1}{ m H}$	$^{13}\mathbf{C}$	$^{15}{ m N}$
Sidechain	57/317 (18%)	39/205 (19%)	18/98 (18%)	0/14 (0%)
Aromatic	8/69 (12%)	4/34 (12%)	4/29 (14%)	0/6 (0%)
Overall	102/585 (17%)	59/319 (18%)	37/211 (18%)	6/55 (11%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 16%, i.e. 117 atoms were assigned a chemical shift out of a possible 724. 0 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathbf{H}$	$^{13}{f C}$	$^{15}{ m N}$
Backbone	42/259~(16%)	18/105 (17%)	17/108~(16%)	7/46 (15%)
Sidechain	$67/386 \ (17\%)$	$46/251 \ (18\%)$	21/120~(18%)	0/15 (0%)
Aromatic	8/79 (10%)	4/39 (10%)	4/34 (12%)	0/6 (0%)
Overall	117/724~(16%)	68/395 (17%)	42/262 (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.

List Id	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
2	P	642	PRO	HD3	1.11	1.76 - 5.48	-6.8
2	Р	641	PRO	HB2	-0.17	0.37 - 3.78	-6.6

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. If well-defined core and ill-defined regions are not identified then it is shown as gray bars.

Random coil index (RCI) for chain P:



