wwPDB NMR Structure Validation Summary Report

Apr 21, 2024 – 04:15 PM EDT

PDB ID	:	2N83
BMRB ID	:	25833
Title	:	p75NTR DD:RIP2 CARD
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Deposited on	:	2015-10-02

This is a wwPDB NMR Structure Validation Summary 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:

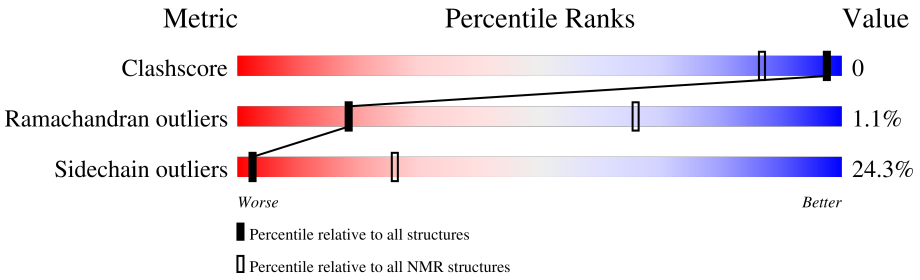
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson 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 84%.

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	98	71% 12% 12% .
2	B	106	69% 18% 12% .

2 Ensemble composition and analysis

This entry contains 10 models. Model 2 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:335-A:351, A:357-A:421, B:435-B:526 (174)	0.70	2

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 2 clusters. No single-model clusters were found.

Cluster number	Models
1	2, 3, 4, 5, 6, 8, 9, 10
2	1, 7

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 3129 atoms, of which 1573 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Tumor necrosis factor receptor superfamily member 16.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	94	1413	444	699	126	142	2	0

- Molecule 2 is a protein called Receptor-interacting serine/threonine-protein kinase 2.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
2	B	105	1716	526	874	146	166	4	0

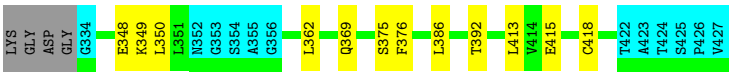
4 Residue-property plots

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: Tumor necrosis factor receptor superfamily member 16

Chain A:



• Molecule 2: Receptor-interacting serine/threonine-protein kinase 2

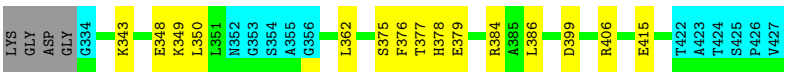
Chain B:

4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 2. Colouring as in section 4.1 above.

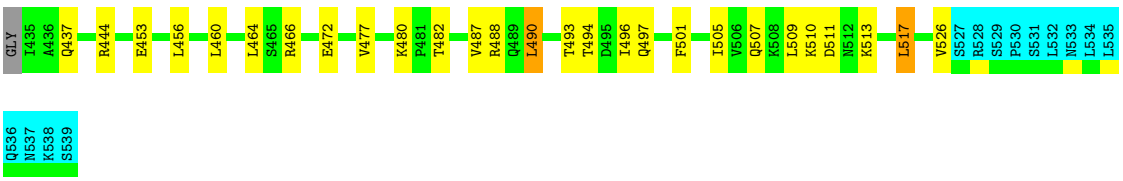
• Molecule 1: Tumor necrosis factor receptor superfamily member 16

Chain A:



• Molecule 2: Receptor-interacting serine/threonine-protein kinase 2

Chain B:



5 Refinement protocol and experimental data overview

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

Of the 100 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
CYANA	structure solution	
Amber	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	2315
Number of shifts mapped to atoms	2278
Number of unparsed shifts	0
Number of shifts with mapping errors	37
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	84%

6 Model quality (i)

6.1 Standard geometry (i)

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	0.69±0.01	0±0/658 (0.0± 0.0%)	0.99±0.02	1±1/897 (0.1± 0.1%)
2	B	0.64±0.01	0±0/750 (0.0± 0.0%)	0.96±0.02	0±0/1013 (0.0± 0.0%)
All	All	0.67	0/14080 (0.0%)	0.98	16/19100 (0.1%)

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	0.2±0.4
2	B	0.0±0.0	0.1±0.3
All	All	0	3

There are no bond-length outliers.

5 of 7 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
1	A	360	ARG	NE-CZ-NH1	6.40	123.50	120.30	4	4
1	A	410	ARG	NE-CZ-NH1	5.51	123.06	120.30	8	3
1	A	406	ARG	NE-CZ-NH1	5.48	123.04	120.30	6	3
2	B	444	ARG	NE-CZ-NH1	5.46	123.03	120.30	10	1
1	A	407	ARG	NE-CZ-NH1	5.43	123.02	120.30	10	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	336	TYR	Sidechain	2

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Mol	Chain	Res	Type	Group	Models (Total)
2	B	466	ARG	Sidechain	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	644	634	634	0±0
2	B	742	768	767	1±1
All	All	13860	14020	14010	14

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.

5 of 9 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:363:ALA:HB2	1:A:386:LEU:HD11	0.66	1.68	9	2
2:B:440:ILE:HG13	2:B:502:ALA:HB1	0.55	1.77	10	1
1:A:405:LEU:HD13	1:A:414:VAL:HG22	0.55	1.79	5	1
2:B:460:LEU:HD21	2:B:477:VAL:HG11	0.54	1.77	3	2
2:B:439:TRP:CZ3	2:B:506:VAL:HG12	0.53	2.38	3	2

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	82/98 (84%)	79±1 (96±1%)	3±1 (3±1%)	1±1 (1±1%)	21	69
2	B	91/106 (86%)	82±2 (90±2%)	8±2 (9±2%)	1±1 (1±1%)	16	63
All	All	1730/2040 (85%)	1604 (93%)	107 (6%)	19 (1%)	18	66

5 of 9 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	396	ALA	5
2	B	513	LYS	4
2	B	518	GLN	2
2	B	524	LEU	2
2	B	441	GLN	2

6.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all NMR entries. The Analysed column shows the number of residues for which the sidechain conformation was analysed and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
1	A	69/78 (88%)	54±2 (78±3%)	15±2 (22±3%)	3	30
2	B	86/99 (87%)	63±2 (74±2%)	23±2 (26±2%)	2	22
All	All	1550/1770 (88%)	1173 (76%)	377 (24%)	2	26

5 of 83 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	349	LYS	10
1	A	386	LEU	10
1	A	415	GLU	10
2	B	482	THR	10
2	B	493	THR	10

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

The completeness of assignment taking into account all chemical shift lists is 84% for the well-defined parts and 84% 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

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	1041
Number of shifts mapped to atoms	1009
Number of unparsed shifts	0
Number of shifts with mapping errors	32
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	6

The following assigned chemical shifts were not mapped to the molecules present in the coordinate file.

- No matching atom found in the structure. First 5 (of 32) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	330	LYS	N	122.586	.	1
1	A	330	LYS	H	8.407	.	1
1	A	330	LYS	CA	53.799	.	1
1	A	330	LYS	HA	4.31	.	1
1	A	330	LYS	CB	30.054	.	1
1	A	330	LYS	HB2	1.83	.	2
1	A	330	LYS	HB3	1.74	.	2
1	A	330	LYS	CG	21.967	.	1
1	A	330	LYS	HG2	1.429	.	2
1	A	330	LYS	HG3	1.377	.	2
1	A	330	LYS	CD	26.302	.	1
1	A	330	LYS	HD2	1.638	.	2
1	A	330	LYS	HD3	1.638	.	2
1	A	330	LYS	CE	39.421	.	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	330	LYS	HE2	2.95	.	2
1	A	330	LYS	HE3	2.95	.	2
1	A	331	GLY	N	109.106	.	1
1	A	331	GLY	H	8.346	.	1
1	A	331	GLY	CA	42.63	.	1
1	A	331	GLY	HA2	3.935	.	2
1	A	332	ASP	N	120.141	.	1
1	A	332	ASP	H	8.198	.	1
1	A	332	ASP	CA	51.533	.	1
1	A	332	ASP	HA	4.586	.	1
1	A	332	ASP	CB	38.524	.	1
1	A	332	ASP	HB2	2.696	.	2
1	A	332	ASP	HB3	2.696	.	2
1	A	333	GLY	N	108.898	.	1
1	A	333	GLY	H	8.44	.	1
1	A	333	GLY	CA	42.861	.	1
1	A	333	GLY	HA2	3.926	.	2
1	A	333	GLY	HA3	3.926	.	2

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	97	2.38 ± 0.14	Should be checked
$^{13}C_{\beta}$	90	3.18 ± 0.10	Should be checked
$^{13}C'$	0	—	None (insufficient data)
^{15}N	93	0.69 ± 0.28	Should be applied

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 38%, i.e. 914 atoms were assigned a chemical shift out of a possible 2426. 0 out of 35 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	315/860 (37%)	156/345 (45%)	81/348 (23%)	78/167 (47%)
Sidechain	555/1453 (38%)	377/944 (40%)	172/453 (38%)	6/56 (11%)
Aromatic	44/113 (39%)	22/56 (39%)	20/51 (39%)	2/6 (33%)
Overall	914/2426 (38%)	555/1345 (41%)	273/852 (32%)	86/229 (38%)

7.1.4 Statistically unusual chemical shifts (i)

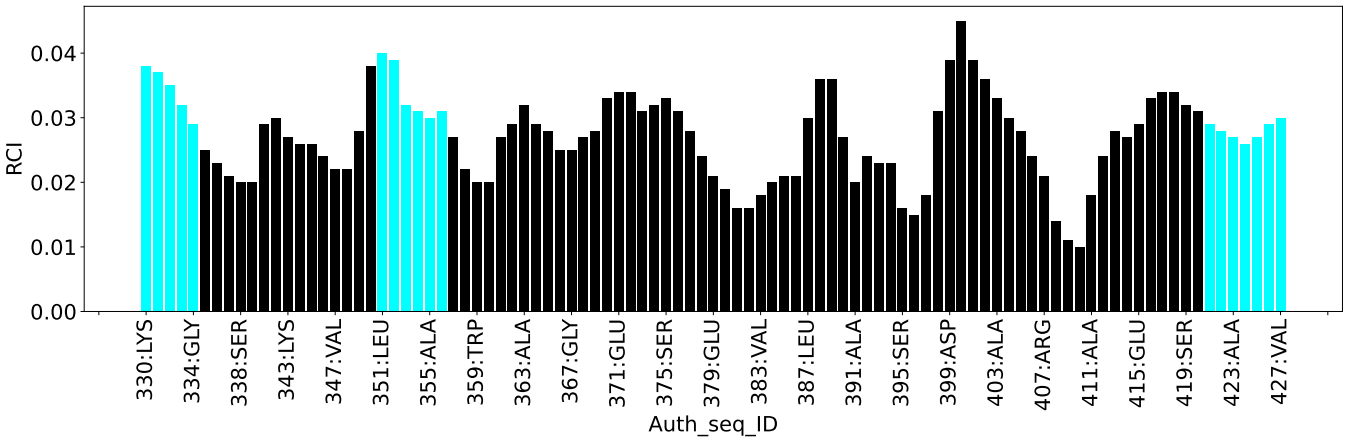
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	396	ALA	HB1	-0.22	0.14 – 2.58	-6.5
1	A	396	ALA	HB2	-0.22	0.14 – 2.58	-6.5
1	A	396	ALA	HB3	-0.22	0.14 – 2.58	-6.5
1	A	382	PRO	HB2	-0.08	0.37 – 3.78	-6.3
1	A	360	ARG	HG2	-0.03	0.26 – 2.87	-6.1
1	A	410	ARG	CD	37.73	38.57 – 47.75	-5.9

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 A:



7.2 Chemical shift list 2

File name: working_cs.cif

Chemical shift list name: *assigned_chem_shift_list_2*

7.2.1 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	1274
Number of shifts mapped to atoms	1269
Number of unparsed shifts	0
Number of shifts with mapping errors	5
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	3

The following assigned chemical shifts were not mapped to the molecules present in the coordinate file.

- No matching atom found in the structure. All 5 occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
2	B	434	GLY	N	112.1	.	1
2	B	434	GLY	H	8.462	.	1
2	B	434	GLY	CA	42.46	.	1
2	B	434	GLY	HA2	3.894	.	2
2	B	434	GLY	HA3	4.19	.	2

7.2.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	106	2.07 ± 0.09	Should be checked
$^{13}C_{\beta}$	103	2.91 ± 0.08	Should be checked
$^{13}C'$	0	—	None (insufficient data)
^{15}N	102	-0.04 ± 0.46	None needed (< 0.5 ppm)

7.2.3 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 46%, i.e. 1125 atoms were assigned a chemical shift out of a possible 2426. 0 out of 35 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	364/860 (42%)	183/345 (53%)	92/348 (26%)	89/167 (53%)

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	Total	^{1}H	^{13}C	^{15}N
Sidechain	749/1453 (52%)	507/944 (54%)	225/453 (50%)	17/56 (30%)
Aromatic	12/113 (11%)	6/56 (11%)	5/51 (10%)	1/6 (17%)
Overall	1125/2426 (46%)	696/1345 (52%)	322/852 (38%)	107/229 (47%)

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	B	471	LYS	HE2	4.20	1.95 – 3.88	6.7
2	B	521	PRO	HD2	1.43	1.93 – 5.38	-6.4
2	B	436	ALA	H	11.72	5.31 – 11.08	6.1

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 B: