



# wwPDB NMR Structure Validation Summary Report ⓘ

Apr 27, 2016 – 12:01 AM BST

PDB ID : 2KXC  
Title : <sup>1</sup>H, <sup>13</sup>C, and <sup>15</sup>N Chemical Shift Assignments for IRTKS-SH3 and EspFu-R47 complex  
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This is a wwPDB NMR Structure Validation Summary Report for a publicly released PDB entry.  
We welcome your comments at [validation@mail.wwpdb.org](mailto: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.

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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

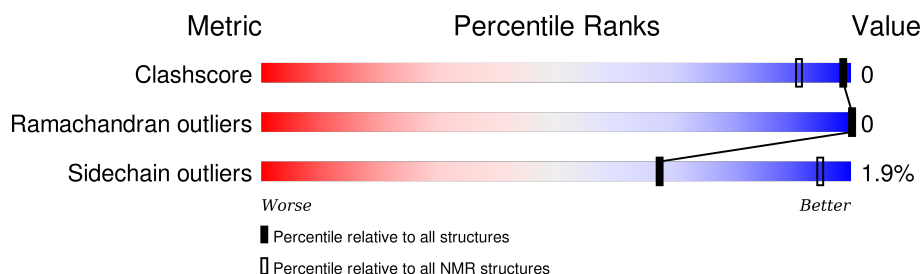
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*SOLUTION NMR*

The overall completeness of chemical shifts assignment is 67%.

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  $\geq 3$ , 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	67	<div> <div>72%</div> <div>28%</div> </div>
2	B	48	<div> <div>27%</div> <div>8%</div> <div>65%</div> </div>

## 2 Ensemble composition and analysis ⓘ

This entry contains 20 models. Model 12 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:344-A:350, A:357-A:397, B:527-B:539 (61)	0.10	12

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	1, 2, 3, 4, 5, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19
2	6, 7, 8, 13, 20

### 3 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 1318 atoms, of which 655 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1.

Mol	Chain	Residues	Atoms						Trace
1	A	67	Total	C	H	N	O	S	0
			1065	344	532	89	99	1	

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	336	GLY	-	EXPRESSION TAG	UNP Q9UHR4
A	337	SER	-	EXPRESSION TAG	UNP Q9UHR4
A	338	HIS	-	EXPRESSION TAG	UNP Q9UHR4

- Molecule 2 is a protein called EspF-like protein.

Mol	Chain	Residues	Atoms					Trace
2	B	17	Total	C	H	N	O	0
			253	86	123	23	21	

There is a discrepancy between the modelled and reference sequences:

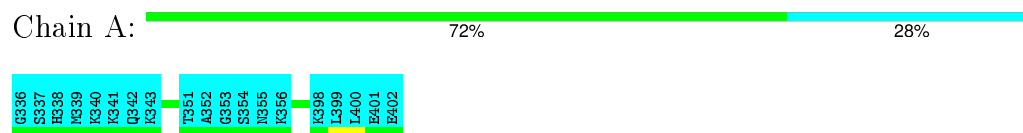
Chain	Residue	Modelled	Actual	Comment	Reference
B	500	GLY	-	EXPRESSION TAG	UNP Q8X2D5

## 4 Residue-property plots [i](#)

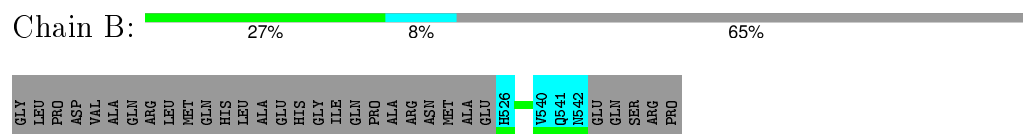
### 4.1 Average score per residue in the NMR ensemble

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: Brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1



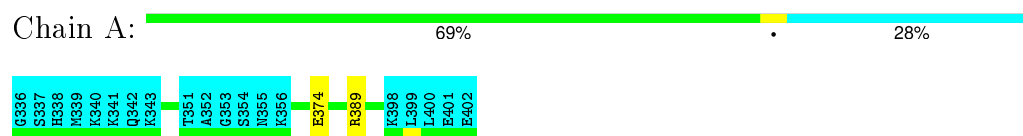
- Molecule 2: EspF-like protein



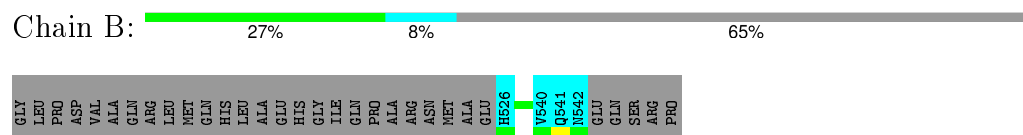
### 4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 12. Colouring as in section 4.1 above.

- Molecule 1: Brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1



- Molecule 2: EspF-like protein



## 5 Refinement protocol and experimental data overview

The models were refined using the following method: *molecular dynamics, TORSION ANGLE DYNAMICS*.

Of the 200 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 2.1	structure solution	2.1
AMBER	refinement	8.0

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)	BMRB entry 16909
Number of chemical shift lists	1
Total number of shifts	924
Number of shifts mapped to atoms	743
Number of unparsed shifts	0
Number of shifts with mapping errors	181
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	67%

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

## 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	0.76±0.00	0±0/401 (0.0±0.0%)	0.95±0.02	1±1/548 (0.1±0.1%)
2	B	0.69±0.01	0±0/105 (0.0±0.0%)	0.98±0.01	0±0/153 (0.0±0.0%)
All	All	0.74	0/10120 (0.0%)	0.96	10/14020 (0.1%)

There are no bond-length outliers.

All unique angle outliers are listed below.

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)	Models	
								Worst	Total
1	A	389	ARG	NE-CZ-NH1	6.50	123.55	120.30	18	10

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	A	387	375	375	0±0
All	All	9660	9360	9360	4

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 0.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:371:ILE:HG22	1:A:372:PRO:HD2	0.67	1.66	16	4

## 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	48/67 (72%)	48±0 (99±1%)	0±0 (1±1%)	0±0 (0±0%)	100	100
2	B	13/48 (27%)	13±0 (100±2%)	0±0 (0±2%)	0±0 (0±0%)	100	100
All	All	1220/2300 (53%)	1214 (100%)	6 (0%)	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	A	42/58 (72%)	41±1 (98±2%)	1±1 (2±2%)	61	93
2	B	11/40 (28%)	11±0 (100±2%)	0±0 (0±2%)	92	98
All	All	1060/1960 (54%)	1040 (98%)	20 (2%)	67	95

5 of 9 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	374	GLU	5
1	A	345	LYS	5
1	A	394	SER	3
1	A	387	LYS	2
1	A	375	LYS	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 67% for the well-defined parts and 63% for the entire structure.

### 7.1 Chemical shift list 1

File name: BMRB entry 16909

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	924
Number of shifts mapped to atoms	743
Number of unparsed shifts	0
Number of shifts with mapping errors	181
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	16

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

- Chain not found in structure. First 5 (of 181) occurrences are reported below.

Chain	Res	Type	Atom	Shift Data		
				Value	Uncertainty	Ambiguity
UNMAPPED	17	ASN	HB2	2.833	0.02	2
UNMAPPED	4	PRO	HA	4.2	0.02	1
UNMAPPED	10	ALA	HB2	-0.015	0.02	1
UNMAPPED	8	TRP	HE1	10.805	0.02	1
UNMAPPED	4	PRO	CD	51.277	0.4	1

#### 7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction $\pm$ precision, ppm	Suggested action
$^{13}\text{C}_\alpha$	82	$-1.37 \pm 0.14$	Should be applied
$^{13}\text{C}_\beta$	76	$-1.34 \pm 0.20$	Should be applied
$^{13}\text{C}'$	0	—	—

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Nucleus	# values	Correction $\pm$ precision, ppm	Suggested action
$^{15}\text{N}$	65	$1.02 \pm 0.77$	None needed (imprecise)

### 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 67%, i.e. 496 atoms were assigned a chemical shift out of a possible 744. 8 out of 8 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^1\text{H}$	$^{13}\text{C}$	$^{15}\text{N}$
Backbone	186/285 (65%)	93/112 (83%)	48/122 (39%)	45/51 (88%)
Sidechain	246/366 (67%)	151/219 (69%)	94/139 (68%)	1/8 (12%)
Aromatic	64/93 (69%)	38/49 (78%)	24/39 (62%)	2/5 (40%)
Overall	496/744 (67%)	282/380 (74%)	166/300 (55%)	48/64 (75%)

### 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.

Mol	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
???	UNMAPPED	6	PRO	HG3	-1.14	3.56 – 0.26	-9.2
???	UNMAPPED	6	PRO	HB2	-0.60	3.82 – 0.32	-7.6
???	UNMAPPED	6	PRO	HB3	-0.70	3.81 – 0.21	-7.5
???	UNMAPPED	6	PRO	HD3	0.93	5.52 – 1.72	-7.1
1	A	380	TYR	HB3	0.21	4.75 – 0.95	-6.9
???	UNMAPPED	5	ALA	HB3	-0.21	2.61 – 0.11	-6.3
???	UNMAPPED	5	ALA	HB2	-0.21	2.61 – 0.11	-6.3
???	UNMAPPED	5	ALA	HB1	-0.21	2.61 – 0.11	-6.3
???	UNMAPPED	5	ALA	HA	1.65	6.46 – 2.06	-5.9
1	A	346	THR	HG21	-0.14	2.29 – -0.01	-5.5
1	A	346	THR	HG23	-0.14	2.29 – -0.01	-5.5
1	A	346	THR	HG22	-0.14	2.29 – -0.01	-5.5
???	UNMAPPED	10	ALA	HB1	-0.01	2.61 – 0.11	-5.5
???	UNMAPPED	10	ALA	HB2	-0.01	2.61 – 0.11	-5.5
???	UNMAPPED	10	ALA	HB3	-0.01	2.61 – 0.11	-5.5
???	UNMAPPED	6	PRO	HG2	0.28	3.48 – 0.38	-5.3

### 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.

Random coil index (RCI) for chain A:

