wwPDB NMR Structure Validation Summary Report (i)

Apr 21, 2024 – 12:45 AM EDT

PDB ID	:	2LLE
BMRB ID	:	18044
Title	:	Computational design of an eight-stranded (beta/alpha)-barrel from fragments of different folds
Authors	:	Coles, M.; Truffault, V.; Eisenbeis, S.; Proffitt, W.; Meiler, J.; Hocker, B.
Deposited on	:	2011-11-07

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:

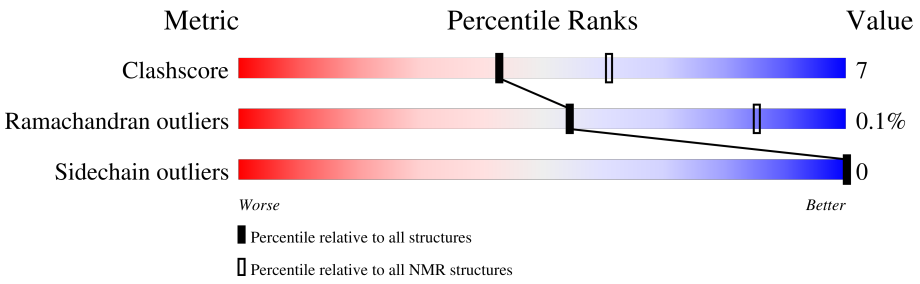
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson 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 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 (#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	234	74% 17% • 7%

2 Ensemble composition and analysis (i)

This entry contains 17 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: *minimized average structure*.

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:3-A:219 (217)	0.23	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 3 clusters and 1 single-model cluster was found.

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

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 3619 atoms, of which 1825 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Chemotaxis protein CheY, Imidazole glycerol phosphate synthase subunit HisF chimera.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	234	3619	1137	1825	310	336	11	0

There are 13 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	4	ILE	ARG	engineered mutation	UNP Q56312
A	78	GLY	ASP	engineered mutation	UNP Q56312
A	95	LEU	ILE	engineered mutation	UNP Q9X0C6
A	201	ALA	LEU	engineered mutation	UNP Q9X0C6
A	213	GLY	VAL	engineered mutation	UNP Q9X0C6
A	227	LEU	-	expression tag	UNP Q9X0C6
A	228	GLU	-	expression tag	UNP Q9X0C6
A	229	HIS	-	expression tag	UNP Q9X0C6
A	230	HIS	-	expression tag	UNP Q9X0C6
A	231	HIS	-	expression tag	UNP Q9X0C6
A	232	HIS	-	expression tag	UNP Q9X0C6
A	233	HIS	-	expression tag	UNP Q9X0C6
A	234	HIS	-	expression tag	UNP Q9X0C6

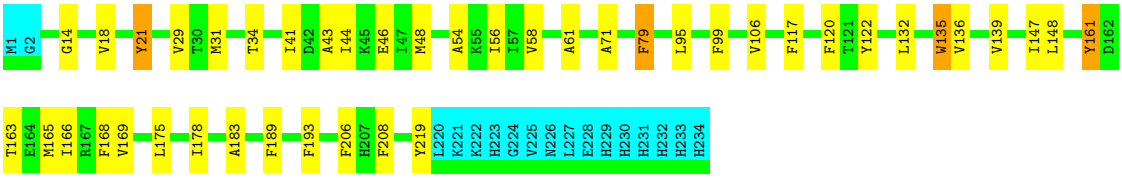
4 Residue-property plots (i)

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: Chemotaxis protein CheY, Imidazole glycerol phosphate synthase subunit HisF chimera

Chain A: 74% 17% • 7%

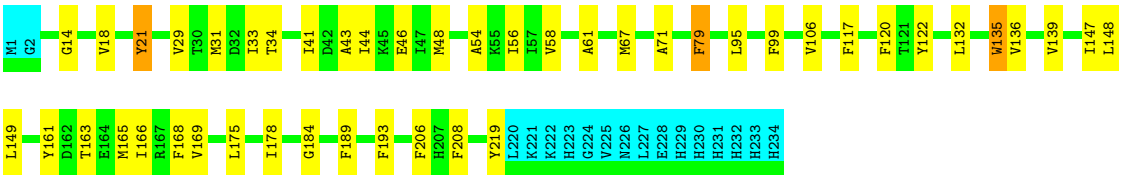


4.2 Residue scores for the representative (medoid) model from the NMR ensemble

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

• Molecule 1: Chemotaxis protein CheY, Imidazole glycerol phosphate synthase subunit HisF chimera

Chain A: 73% 18% • 7%



5 Refinement protocol and experimental data overview (i)

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

Of the 50 calculated structures, 17 were deposited, based on the following criterion: *structures with the least restraint violations*.

The following table shows the software used for structure solution, optimisation and refinement.

Software name	**Classification**	**Version**
X-PLOR NIH	structure solution	2.21
X-PLOR NIH	refinement	2.21

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	1
Total number of shifts	2699
Number of shifts mapped to atoms	2699
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)

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	1.36±0.00	64±0/1672 (3.8± 0.0%)	0.74±0.00	0±0/2256 (0.0± 0.0%)
All	All	1.36	1088/28424 (3.8%)	0.74	0/38352 (0.0%)

5 of 64 unique bond 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(Å)	Ideal(Å)	Models	
								Worst	Total
1	A	122	TYR	CE2-CZ	6.96	1.47	1.38	6	17
1	A	219	TYR	CE1-CZ	6.95	1.47	1.38	16	17
1	A	219	TYR	CE2-CZ	6.94	1.47	1.38	10	17
1	A	122	TYR	CE1-CZ	6.89	1.47	1.38	9	17
1	A	21	TYR	CE2-CZ	6.89	1.47	1.38	11	17

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	A	1649	1690	1690	24±2
All	All	28033	28730	28730	405

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 7.

5 of 62 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:79:PHE:CZ	1:A:95:LEU:HD22	0.72	2.20	1	16
1:A:132:LEU:O	1:A:136:VAL:HG23	0.71	1.86	11	17
1:A:135:TRP:O	1:A:139:VAL:HG23	0.70	1.86	17	17
1:A:44:ILE:HG23	1:A:56:ILE:HG21	0.69	1.65	11	17
1:A:106:VAL:HG21	1:A:139:VAL:HG13	0.68	1.65	6	17

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	217/234 (93%)	213±1 (98±0%)	3±1 (2±0%)	0±0 (0±0%)	54	85
All	All	3689/3978 (93%)	3628 (98%)	57 (2%)	4 (0%)	54	85

All 1 unique Ramachandran outliers are listed below.

Mol	Chain	Res	Type	Models (Total)
1	A	184	GLY	4

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	173/188 (92%)	173±0 (100±0%)	0±0 (0±0%)	100	100
All	All	2941/3196 (92%)	2941 (100%)	0 (0%)	100	100

There are no protein residues with a non-rotameric sidechain to report.

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 85% 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	2699
Number of shifts mapped to atoms	2699
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	4

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	225	-0.40 ± 0.06	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	205	0.01 ± 0.12	None needed (< 0.5 ppm)
$^{13}C'$	189	-0.20 ± 0.10	None needed (< 0.5 ppm)
^{15}N	205	0.82 ± 0.27	Should be applied

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 88%, i.e. 2573 atoms were assigned a chemical shift out of a possible 2916. 0 out of 31 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	1022/1091 (94%)	429/446 (96%)	397/434 (91%)	196/211 (93%)
Sidechain	1479/1673 (88%)	1008/1097 (92%)	461/522 (88%)	10/54 (19%)

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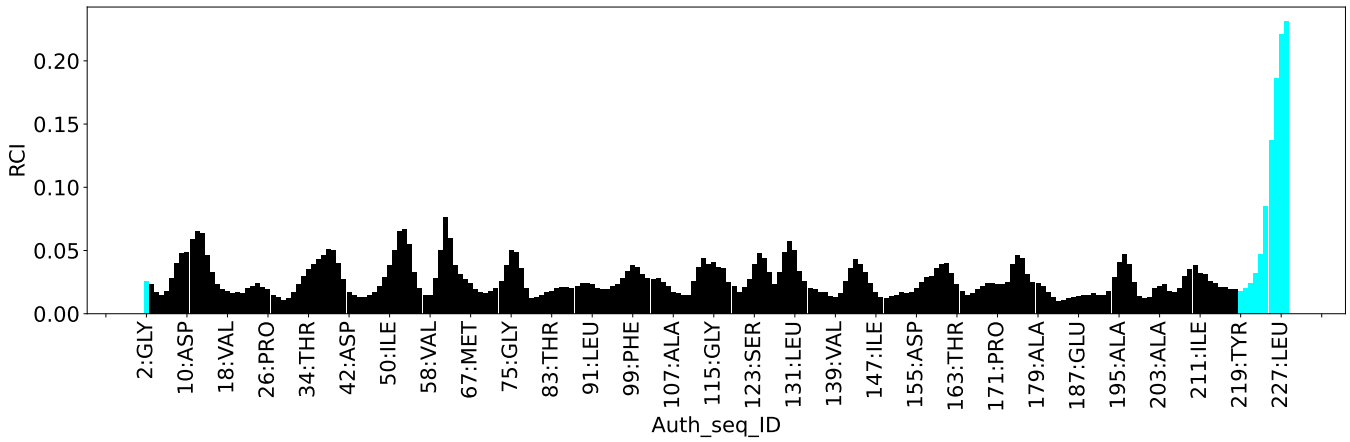
	Total	^{1}H	^{13}C	^{15}N
Aromatic	72/152 (47%)	65/75 (87%)	6/74 (8%)	1/3 (33%)
Overall	2573/2916 (88%)	1502/1618 (93%)	864/1030 (84%)	207/268 (77%)

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	173	THR	HG1	5.89	0.08 – 2.19	22.5
1	A	128	THR	HG1	5.44	0.08 – 2.19	20.4
1	A	93	THR	HG1	4.70	0.08 – 2.19	16.9
1	A	160	GLY	HA2	1.76	2.15 – 5.77	-6.1

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: