wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	2L48
Title	:	Solution structure of the PlyG cell wall binding domain
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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
A user guide is available at
http://wwpdb.org/validation/2016/NMRValidationReportHelp
with specific help available everywhere you see the (i) symbol.

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

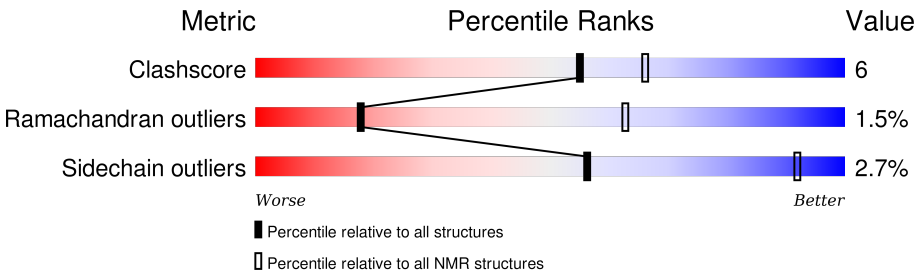
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	unknown
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	rb-20027457
Ideal 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 (i)

The following experimental techniques were used to determine the structure:
SOLUTION NMR

The overall completeness of chemical shifts assignment is 93%.

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 >=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	85	62% 14% • 22%
1	B	85	64% 13% • 22%

2 Ensemble composition and analysis (i)

This entry contains 20 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:168-A:233, B:368-B:433 (132)	0.25	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 and 4 single-model clusters were found.

Cluster number	Models
1	4, 8, 9, 10, 13, 15, 16, 17, 19
2	1, 2, 5, 6, 12, 18, 20
Single-model clusters	3; 7; 11; 14

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 2477 atoms, of which 1150 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called N-acetylmuramoyl-L-alanine amidase.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	85	1239	424	576	107	129	3	0
1	B	85	1238	424	574	107	130	3	0

There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	149	GLY	-	EXPRESSION TAG	UNP Q8LTE6
A	150	SER	-	EXPRESSION TAG	UNP Q8LTE6
B	349	GLY	-	EXPRESSION TAG	UNP Q8LTE6
B	350	SER	-	EXPRESSION TAG	UNP Q8LTE6

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: N-acetylmuramoyl-L-alanine amidase

Chain A: 62% 14% • 22%

G149 S150 I151 Q152 K153 V154 K155 N156 G157 N158 V159 A160 T161 T162 S163 P164 T165 K166 Q167 N168 I169 I170 Q171 T180 P181 T193 L198 I207 S208 K217 K220 E221 R225 K233

- Molecule 1: N-acetylmuramoyl-L-alanine amidase

Chain B: 64% 13% • 22%

G349 S350 I351 Q352 K353 V354 K355 N356 G357 N358 V359 A360 T361 T362 S363 P364 T365 K366 Q367 N368 I369 I370 Q371 T380 P381 T393 L398 I407 S408 K420 E421 R425 K433

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: N-acetylmuramoyl-L-alanine amidase

Chain A: 59% 18% • 22%

G149 S150 I151 Q152 K153 V154 K155 N156 G157 N158 V159 A160 T161 T162 S163 P164 T165 K166 Q167 N168 I169 I170 Q171 T180 P181 T188 K191 M192 T193 L198 I207 S208 K209 P210 A214 K217 E221 R225 K233

- Molecule 1: N-acetylmuramoyl-L-alanine amidase

Chain B: 64% 13% • 22%

G349 S350 I351 Q352 K353 V354 K355 N356 G357 N358 V359 A360 T361 T362 S363 P364 T365 K366 Q367 N368 I369 T380 P381 T388 K391 M392 T393 I397 L398 S408 E421 R425 K433

5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *AUTOMATED METHODS WERE USED FOR BACKBONE CHEMICAL SHIFT ASSIGNMENT AND ITERATIVE NOE REFINEMENT. FINAL STRUCTURES WERE OBTAINED BY MOLECULAR DYNAMICS IN EXPLICIT SOLVENT, AUTOMATED METHODS WERE USED FOR BACKBONE CHEMICAL SHIFT ASSIGNMENT AND ITERATIVE NOE REFINEMENT. FINAL STRUCTURES WERE OBTAINED BY MOLECULAR DYNAMICS IN EXPLICIT SOLVENT.*

Of the 100 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**
Xplor-NIH	refinement	2.9.3

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 17233
Number of chemical shift lists	1
Total number of shifts	2124
Number of shifts mapped to atoms	2124
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	93%

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)

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	A	527	452	520	8±2
1	B	528	452	520	7±2
All	All	21100	18080	20800	272

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.

5 of 72 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:193:THR:HG23	1:B:398:LEU:O	0.68	1.88	15	11
1:A:198:LEU:O	1:B:393:THR:HG23	0.63	1.93	9	13
1:B:421:GLU:O	1:B:425:ARG:HG3	0.62	1.95	4	6
1:A:221:GLU:O	1:A:225:ARG:HG2	0.60	1.97	12	12
1:B:417:LYS:O	1:B:421:GLU:HG2	0.59	1.98	13	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	65/85 (76%)	63±1 (96±1%)	1±1 (2±1%)	1±0 (2±0%)	18	63
1	B	65/85 (76%)	63±1 (96±1%)	2±1 (2±1%)	1±0 (1±0%)	18	63
All	All	2600/3400 (76%)	2503 (96%)	58 (2%)	39 (2%)	18	63

All 3 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	193	THR	19
1	B	393	THR	19
1	A	168	ASN	1

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	57/73 (78%)	56±1 (97±1%)	2±1 (3±1%)	57	92
1	B	57/73 (78%)	55±1 (97±2%)	2±1 (3±2%)	56	92
All	All	2280/2920 (78%)	2219 (97%)	61 (3%)	56	92

5 of 16 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	220	LYS	11
1	B	420	LYS	11
1	B	409	LYS	7
1	A	209	LYS	6
1	A	217	LYS	5

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 93% for the well-defined parts and 92% for the entire structure.

7.1 Chemical shift list 1

File name: BMRB entry 17233

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	2124
Number of shifts mapped to atoms	2124
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
$^{13}C_{\alpha}$	164	-0.57 ± 0.13	Should be applied
$^{13}C_{\beta}$	154	-0.24 ± 0.14	None needed (< 0.5 ppm)
$^{13}C'$	164	-0.43 ± 0.16	None needed (< 0.5 ppm)
^{15}N	154	0.35 ± 0.23	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 93%, i.e. 1498 atoms were assigned a chemical shift out of a possible 1618. 14 out of 16 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	634/648 (98%)	254/258 (98%)	258/264 (98%)	122/126 (97%)
Sidechain	726/804 (90%)	456/474 (96%)	260/304 (86%)	10/26 (38%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	138/166 (83%)	86/86 (100%)	48/76 (63%)	4/4 (100%)
Overall	1498/1618 (93%)	796/818 (97%)	566/644 (88%)	136/156 (87%)

7.1.4 Statistically unusual chemical shifts (i)

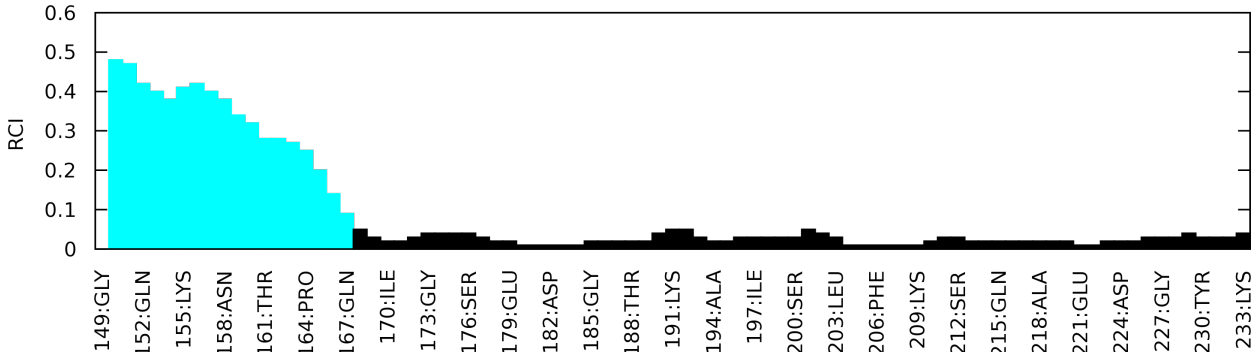
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	B	409	LYS	CE	50.28	46.00 – 37.80	10.2
1	A	209	LYS	CE	50.28	46.00 – 37.80	10.2
1	B	426	LYS	HB3	0.09	3.10 – 0.40	-6.2
1	A	226	LYS	HB3	0.09	3.10 – 0.40	-6.2

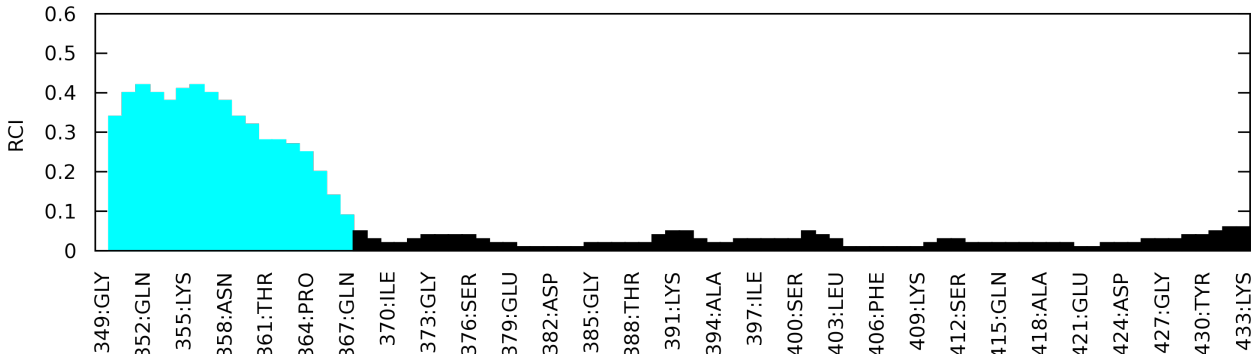
7.1.5 Random Coil Index (RCI) plots (i)

The images below report *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:



Random coil index (RCI) for chain B:


RCI
0.6
0.5
0.4
0.3
0.2
0.1
0
349:GLY
352:GLN
355:LYS
358:ASN
361:THR
364:PRO
367:GLN
370:ILE
373:GLY
376:SER
379:GLU
382:ASP
385:GLY
388:THR
391:LYS
394:ALA
397:ILE
400:SER
403:LEU
406:PHE
409:LYS
412:SER
415:GLN
418:ALA
421:GLU
424:ASP
427:GLY
430:TYR
433:LYS