wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	6M78
Title	:	Aromatic interactions drive the coupled folding and binding of the intrinsically disordered Sesbania mosaic virus VPg protein
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Deposited on	:	2020-03-17

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

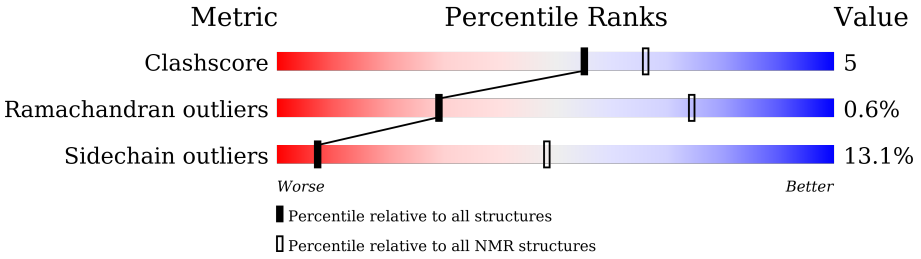
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	2.16
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.16

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 29%.

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	296	50% 14% 35%
2	B	80	34% 29% • 8% 29%

2 Ensemble composition and analysis (i)

This entry contains 4 models. Model 4 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:135-A:325, B:323-B:341, B:348-B:379 (242)	0.36	4

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 1 clusters. No single-model clusters were found.

Cluster number	Models
1	1, 2, 3, 4

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 2304 atoms, of which 414 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Polyprotein.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	191	1746	899	319	241	274	13	0

There are 13 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	107	MET	-	initiating methionine	UNP Q9EB08
A	108	ARG	-	expression tag	UNP Q9EB08
A	109	GLY	-	expression tag	UNP Q9EB08
A	110	SER	-	expression tag	UNP Q9EB08
A	111	HIS	-	expression tag	UNP Q9EB08
A	112	HIS	-	expression tag	UNP Q9EB08
A	113	HIS	-	expression tag	UNP Q9EB08
A	114	HIS	-	expression tag	UNP Q9EB08
A	115	HIS	-	expression tag	UNP Q9EB08
A	116	HIS	-	expression tag	UNP Q9EB08
A	117	GLY	-	expression tag	UNP Q9EB08
A	118	MET	-	expression tag	UNP Q9EB08
A	119	ALA	-	expression tag	UNP Q9EB08

- Molecule 2 is a protein called Polyprotein.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
2	B	57	558	300	95	73	89	1	0

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	323	GLY	-	expression tag	UNP Q9EB08
B	324	SER	-	expression tag	UNP Q9EB08
B	325	MET	-	expression tag	UNP Q9EB08

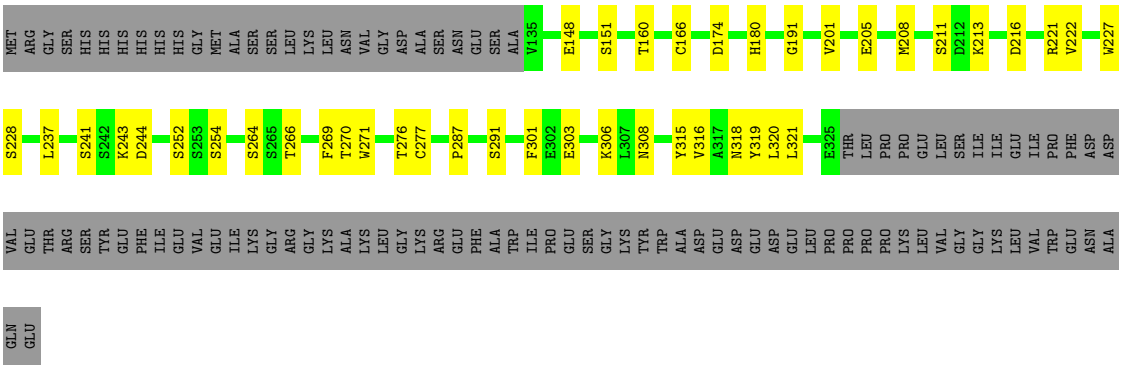
4 Residue-property plots (i)

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.

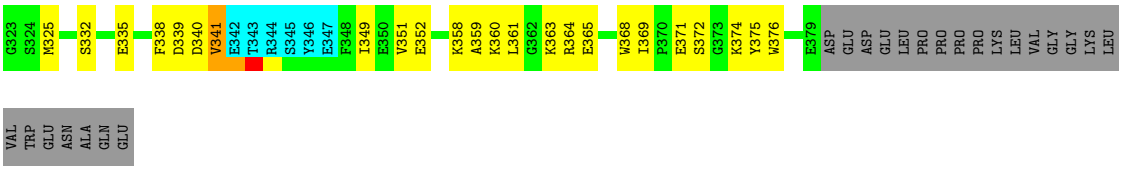
• Molecule 1: Polyprotein

Chain A: 50% 14% 35%



• Molecule 2: Polyprotein

Chain B: 34% 29% • 8% 29%

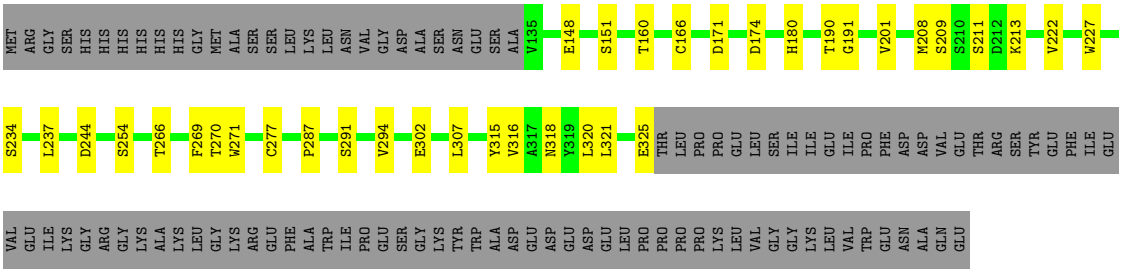


4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 4. Colouring as in section 4.1 above.

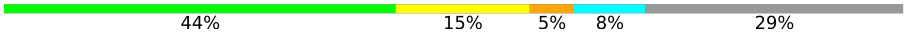
• Molecule 1: Polyprotein

Chain A: 52% 12% 35%



- Molecule 2: Polyprotein

Chain B:



5 Refinement protocol and experimental data overview (i)

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

Of the 200 calculated structures, 4 were deposited, based on the following criterion: *structures with favorable non-bond energy*.

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

Software name	**Classification**	**Version**
HADDOCK	structure calculation	2.2
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	1
Total number of shifts	1000
Number of shifts mapped to atoms	1000
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	29%

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	0.29±0.00	0±0/1461 (0.0± 0.0%)	0.42±0.00	0±0/1982 (0.0± 0.0%)
2	B	0.31±0.00	0±0/420 (0.0± 0.0%)	0.96±0.03	3±0/566 (0.5± 0.1%)
All	All	0.29	0/7524 (0.0%)	0.59	11/10192 (0.1%)

There are no bond-length outliers.

All 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
2	B	341	VAL	CB-CA-C	-18.11	77.00	111.40	4	4
2	B	341	VAL	N-CA-C	-6.88	92.43	111.00	2	4
2	B	341	VAL	N-CA-CB	6.24	125.24	111.50	4	3

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	1427	319	1403	12±2
2	B	409	81	406	9±2
All	All	7344	1600	7236	78

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 5.

5 of 49 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
2:B:349:ILE:HB	2:B:361:LEU:HB2	0.92	1.40	1	3
2:B:359:ALA:HB1	2:B:368:TRP:HB2	0.88	1.45	2	3
1:A:237:LEU:HG	1:A:294:VAL:HB	0.73	1.59	4	1
1:A:316:VAL:HA	1:A:320:LEU:HB2	0.69	1.65	4	3
1:A:317:ALA:HA	1:A:321:LEU:HD12	0.68	1.65	2	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	Percentiles	
1	A	189/296 (64%)	176±2 (93±1%)	13±2 (7±1%)	1±1 (0±0%)	44	80
2	B	49/80 (61%)	42±1 (85±2%)	6±1 (13±2%)	1±1 (2±1%)	11	52
All	All	952/1504 (63%)	869 (91%)	77 (8%)	6 (1%)	29	74

All 4 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
2	B	376	TRP	2
2	B	332	SER	2
1	A	181	HIS	1
1	A	182	VAL	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	161/250 (64%)	141±3 (87±2%)	20±3 (13±2%)	8	50

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
2	B	43/69 (62%)	37±1 (85±3%)	7±1 (15±3%)	6 44
All	All	816/1276 (64%)	709 (87%)	107 (13%)	7 48

5 of 62 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	174	ASP	4
1	A	271	TRP	4
2	B	335	GLU	4
1	A	291	SER	4
1	A	270	THR	4

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 29% for the well-defined parts and 28% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *assigned_chemical_shifts_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	1000
Number of shifts mapped to atoms	1000
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	1

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	250	0.41 ± 0.09	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	211	0.73 ± 0.17	Should be applied
$^{13}C'$	0	—	None (insufficient data)
^{15}N	215	0.12 ± 0.31	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 29%, i.e. 806 atoms were assigned a chemical shift out of a possible 2818. 0 out of 37 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	585/1184 (49%)	185/471 (39%)	215/484 (44%)	185/229 (81%)
Sidechain	217/1384 (16%)	33/816 (4%)	184/522 (35%)	0/46 (0%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	4/250 (2%)	2/131 (2%)	0/105 (0%)	2/14 (14%)
Overall	806/2818 (29%)	220/1418 (16%)	399/1111 (36%)	187/289 (65%)

7.1.4 Statistically unusual chemical shifts (i)

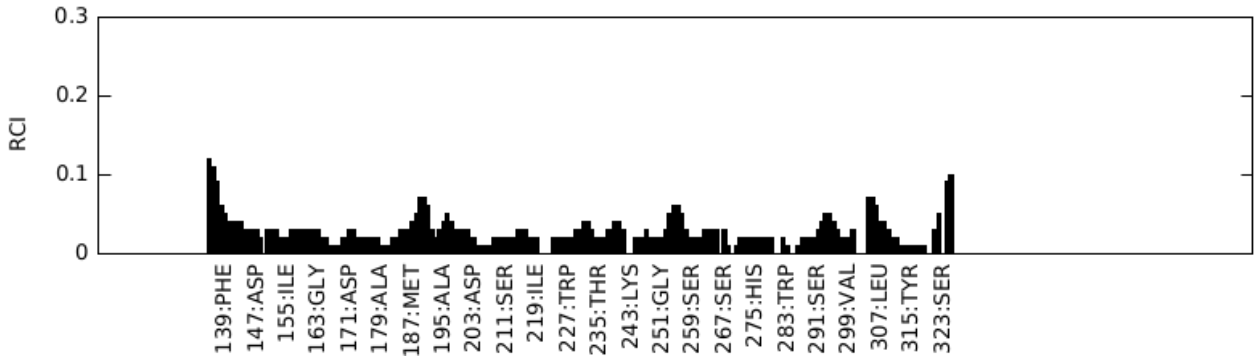
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	268	GLU	CB	38.91	38.65 – 21.35	5.1

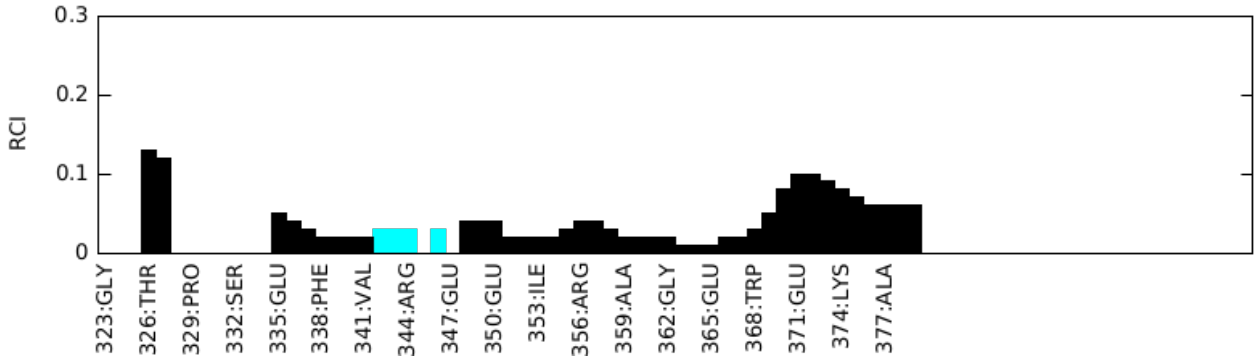
7.1.5 Random Coil Index (RCI) plots (i)

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
0.3
0.2
0.1
0
323:GLY
326:THR
329:PRO
332:SER
335:GLU
338:PHE
341:VAL
344:ARG
347:GLU
350:GLU
353:ILE
356:ARG
359:ALA
362:GLY
365:GLU
368:TRP
371:GLU
374:LYS
377:ALA