Full wwPDB NMR Structure Validation Report (i)

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PDB ID	:	2L9V
BMRB ID	:	17489
Title	:	NMR structure of the FF domain L24A mutant's folding transition state
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This is a Full wwPDB NMR Structure Validation 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:

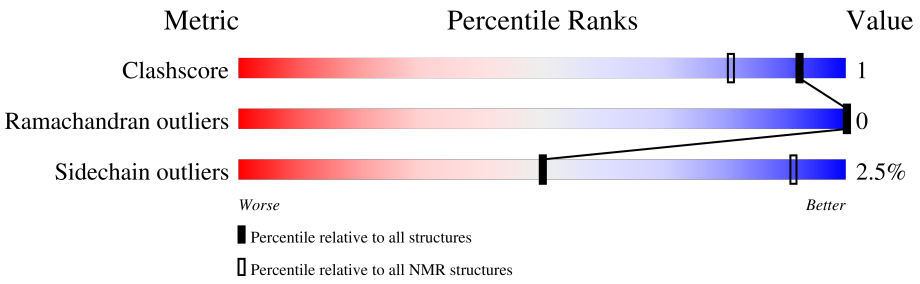
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson 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 32%.

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	70	53% · 16% 30%

2 Ensemble composition and analysis (i)

This entry contains 9 models. Model 6 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:17-A:54 (38)	0.64	6

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 2 single-model clusters were found.

Cluster number	Models
1	1, 4, 5, 6, 7
2	2, 3
Single-model clusters	8; 9

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 814 atoms, of which 413 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Pre-mRNA-processing factor 40 homolog A.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	49	814	254	413	71	74	2	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	24	ALA	LEU	engineered mutation	UNP O75400

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: Pre-mRNA-processing factor 40 homolog A

Chain A: 53% • 16% 30%

SER GLN PRO ALA LYS LYS THR TYR THR W11 N12 T13 K14 E15 E16 L25 L55 S56 E57 K58 K59 GLN ALA PHE ASN ALA TYR LYS VAL GLN THR GLU LYS

4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

• Molecule 1: Pre-mRNA-processing factor 40 homolog A

Chain A: 53% • 16% 30%

SER GLN PRO ALA LYS LYS THR TYR THR W11 N12 T13 K14 E15 E16 L25 L55 S56 E57 K58 K59 GLN ALA PHE ASN ALA TYR LYS VAL GLN THR GLU LYS

4.2.2 Score per residue for model 2

• Molecule 1: Pre-mRNA-processing factor 40 homolog A

Chain A: 53% • 16% 30%

SER GLN PRO ALA LYS LYS THR TYR THR W11 N12 T13 K14 E15 E16 L25 L55 S56 E57 K58 K59 GLN ALA PHE ASN ALA TYR LYS VAL GLN THR GLU LYS

4.2.3 Score per residue for model 3

• Molecule 1: Pre-mRNA-processing factor 40 homolog A

Chain A: 53% • 16% 30%

SER GLN PRO ALA LYS LYS THR TYR THR W11 N12 T13 K14 E15 E16 L25 L55 S56 E57 K58 K59 GLN ALA PHE ASN ALA TYR LYS VAL GLN THR GLU LYS

4.2.4 Score per residue for model 4

• Molecule 1: Pre-mRNA-processing factor 40 homolog A

Chain A: 53% • 16% 30%

SER GLN PRO ALA LYS LYS THR TYR THR W11 N12 T13 K14 E15 E16 L25 L55 S56 E57 K58 K59 GLN ALA PHE ASN ALA TYR LYS VAL GLN THR GLU LYS

4.2.5 Score per residue for model 5

• Molecule 1: Pre-mRNA-processing factor 40 homolog A

Chain A: 53% • 16% 30%

SER GLN PRO ALA LYS LYS THR TYR THR W11 N12 T13 K14 E15 E16 L25 L55 S56 E57 K58 K59 GLN ALA PHE ASN ALA TYR LYS VAL GLN THR GLU LYS

4.2.6 Score per residue for model 6 (medoid)

• Molecule 1: Pre-mRNA-processing factor 40 homolog A

Chain A: 51% • 16% 30%

SER GLN PRO ALA LYS LYS THR TYR THR W11 N12 T13 K14 E15 E16 L25 K28 L55 S56 E57 K58 K59 GLN ALA PHE ASN ALA TYR LYS VAL GLN THR GLU LYS

4.2.7 Score per residue for model 7

• Molecule 1: Pre-mRNA-processing factor 40 homolog A

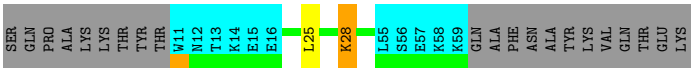
Chain A: 51% • 16% 30%

SER GLN PRO ALA LYS LYS THR TYR THR W11 N12 T13 K14 E15 E16 L25 K28 L55 S56 E57 K58 K59 GLN ALA PHE ASN ALA TYR LYS VAL GLN THR GLU LYS

4.2.8 Score per residue for model 8

- Molecule 1: Pre-mRNA-processing factor 40 homolog A

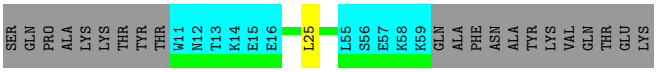
Chain A:



4.2.9 Score per residue for model 9

- Molecule 1: Pre-mRNA-processing factor 40 homolog A

Chain A:



5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *Monte Carlo*.

Of the 10000 calculated structures, 9 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
CSRosetta	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	309
Number of shifts mapped to atoms	219
Number of unparsed shifts	0
Number of shifts with mapping errors	90
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	32%

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	304	317	317	1±1
All	All	2736	2853	2853	8

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.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:25:LEU:C	1:A:25:LEU:HD23	0.53	2.24	9	3
1:A:28:LYS:HE2	1:A:28:LYS:HA	0.52	1.80	6	2
1:A:28:LYS:O	1:A:28:LYS:HG3	0.51	2.06	8	1
1:A:25:LEU:HD23	1:A:25:LEU:O	0.48	2.08	9	1
1:A:28:LYS:HE3	1:A:28:LYS:HA	0.43	1.88	8	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	38/70 (54%)	38±0 (100±0%)	0±0 (0±0%)	0±0 (0±0%)	100	100
All	All	342/630 (54%)	342 (100%)	0 (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	31/60 (52%)	30±0 (97±1%)	1±0 (3±1%)	50	91
All	All	279/540 (52%)	272 (97%)	7 (3%)	50	91

All 2 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	25	LEU	6
1	A	28	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 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 32% for the well-defined parts and 32% 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	309
Number of shifts mapped to atoms	219
Number of unparsed shifts	0
Number of shifts with mapping errors	90
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	0

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

- No matching atom found in the structure. All 90 occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	2	SER	C	174.276	.	2
1	A	3	GLN	H	8.446	.	2
1	A	3	GLN	HA	4.671	.	2
1	A	3	GLN	CA	53.944	.	2
1	A	3	GLN	N	122.894	.	2
1	A	4	PRO	HA	4.42	.	2
1	A	4	PRO	C	176.649	.	2
1	A	4	PRO	CA	63.389	.	2
1	A	5	ALA	H	8.358	.	2
1	A	5	ALA	HA	4.285	.	2
1	A	5	ALA	C	177.82	.	2
1	A	5	ALA	CA	52.373	.	2
1	A	5	ALA	N	124.657	.	2
1	A	6	LYS	H	8.203	.	2

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	6	LYS	HA	4.291	.	2
1	A	6	LYS	C	176.515	.	2
1	A	6	LYS	CA	56.215	.	2
1	A	6	LYS	N	121.077	.	2
1	A	7	LYS	H	8.294	.	2
1	A	7	LYS	C	176.32	.	2
1	A	7	LYS	N	123.386	.	2
1	A	8	THR	H	8.009	.	2
1	A	8	THR	HA	4.242	.	2
1	A	8	THR	C	173.78	.	2
1	A	8	THR	CA	61.576	.	2
1	A	8	THR	N	115.881	.	2
1	A	9	TYR	H	8.179	.	2
1	A	9	TYR	HA	4.545	.	2
1	A	9	TYR	C	175.479	.	2
1	A	9	TYR	CA	57.582	.	2
1	A	9	TYR	N	122.622	.	2
1	A	10	THR	H	7.862	.	2
1	A	10	THR	HA	4.224	.	2
1	A	10	THR	CA	61.57	.	2
1	A	10	THR	N	115.758	.	2
1	A	60	GLN	H	8.254	.	2
1	A	60	GLN	HA	4.255	.	2
1	A	60	GLN	C	176.011	.	2
1	A	60	GLN	CA	56.794	.	2
1	A	60	GLN	N	121.104	.	2
1	A	61	ALA	H	8.231	.	2
1	A	61	ALA	HA	4.3	.	2
1	A	61	ALA	C	177.717	.	2
1	A	61	ALA	CA	52.834	.	2
1	A	61	ALA	N	124.802	.	2
1	A	62	PHE	H	7.993	.	2
1	A	62	PHE	HA	4.707	.	2
1	A	62	PHE	C	175.564	.	2
1	A	62	PHE	CA	58.16	.	2
1	A	62	PHE	N	119.423	.	2
1	A	63	ASN	H	8.107	.	2
1	A	63	ASN	HA	4.564	.	2
1	A	63	ASN	CA	53.382	.	2
1	A	63	ASN	N	120.572	.	2
1	A	64	ALA	H	7.983	.	2

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	64	ALA	HA	4.189	.	2
1	A	64	ALA	C	177.56	.	2
1	A	64	ALA	CA	52.942	.	2
1	A	64	ALA	N	124.013	.	2
1	A	65	TYR	H	7.884	.	2
1	A	65	TYR	HA	4.483	.	2
1	A	65	TYR	C	175.714	.	2
1	A	65	TYR	CA	58.003	.	2
1	A	65	TYR	N	118.567	.	2
1	A	66	LYS	H	7.838	.	2
1	A	66	LYS	HA	4.287	.	2
1	A	66	LYS	C	176.091	.	2
1	A	66	LYS	CA	56.331	.	2
1	A	66	LYS	N	123.002	.	2
1	A	67	VAL	H	8.028	.	2
1	A	67	VAL	HA	4.048	.	2
1	A	67	VAL	C	176.271	.	2
1	A	67	VAL	N	121.481	.	2
1	A	68	GLN	H	8.407	.	2
1	A	68	GLN	C	176.014	.	2
1	A	68	GLN	CA	55.833	.	2
1	A	68	GLN	N	124.246	.	2
1	A	69	THR	H	8.116	.	2
1	A	69	THR	HA	4.319	.	2
1	A	69	THR	C	174.445	.	2
1	A	69	THR	CA	61.701	.	2
1	A	69	THR	N	115.678	.	2
1	A	70	GLU	H	8.346	.	2
1	A	70	GLU	C	175.606	.	2
1	A	70	GLU	CA	56.337	.	2
1	A	70	GLU	N	123.699	.	2
1	A	71	LYS	H	7.599	.	2
1	A	71	LYS	HA	4.146	.	2
1	A	71	LYS	CA	57.59	.	2
1	A	71	LYS	N	126.692	.	2

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	60	0.32 ± 0.26	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	0	—	None (insufficient data)
$^{13}C'$	60	0.23 ± 0.16	None needed (< 0.5 ppm)
^{15}N	66	-0.77 ± 0.14	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 32%, i.e. 171 atoms were assigned a chemical shift out of a possible 536. 0 out of 3 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	171/186 (92%)	68/74 (92%)	67/76 (88%)	36/36 (100%)
Sidechain	0/319 (0%)	0/206 (0%)	0/97 (0%)	0/16 (0%)
Aromatic	0/31 (0%)	0/15 (0%)	0/15 (0%)	0/1 (0%)
Overall	171/536 (32%)	68/295 (23%)	67/188 (36%)	36/53 (68%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 32%, i.e. 219 atoms were assigned a chemical shift out of a possible 695. 0 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	219/241 (91%)	87/96 (91%)	85/98 (87%)	47/47 (100%)
Sidechain	0/411 (0%)	0/263 (0%)	0/128 (0%)	0/20 (0%)
Aromatic	0/43 (0%)	0/21 (0%)	0/20 (0%)	0/2 (0%)
Overall	219/695 (32%)	87/380 (23%)	85/246 (35%)	47/69 (68%)

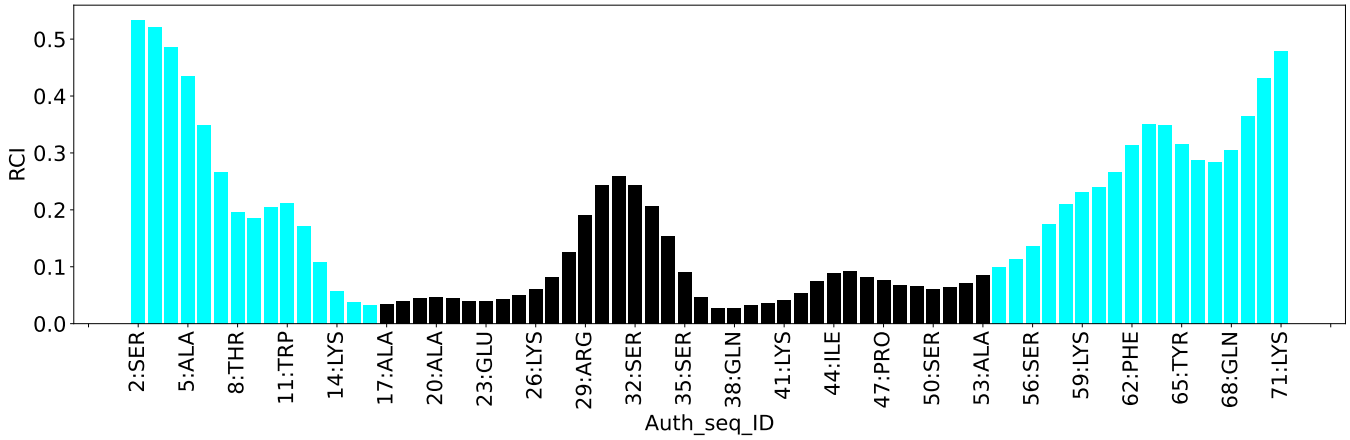
7.1.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

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:


RCI
0.0
0.1
0.2
0.3
0.4
0.5
2:SER
5:ALA
8:THR
11:TRP
14:LYS
17:ALA
20:ALA
23:GLU
26:LYS
29:ARG
32:SER
35:SER
38:GLN
41:LYS
44:ILE
47:PRO
50:SER
53:ALA
56:SER
59:LYS
62:PHE
65:TYR
68:GLN
71:LYS
Auth_seq_ID