wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	2RUE
Title	:	Solution structure of the a' domain of thermophilic fungal protein disulfide (oxidized form, 303K)
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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:

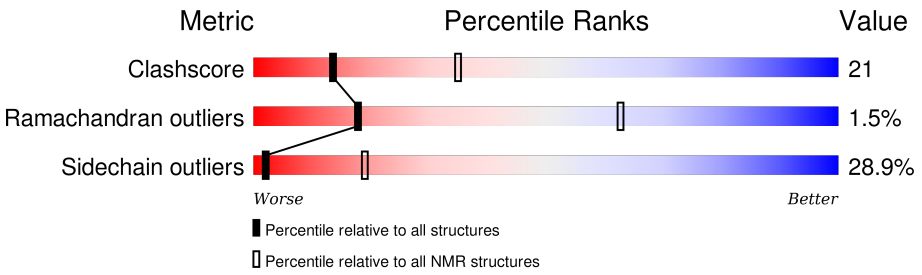
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	unknown
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	rb-20027457
Ideal 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 85%.

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	121	47% 36% 8% 9%

2 Ensemble composition and analysis (i)

This entry contains 10 models. Model 1 is the overall representative, medoid model (most similar to other models).

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:9-A:118 (110)	0.29	1

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 2 single-model clusters were found.

Cluster number	Models
1	2, 5, 7
2	4, 6, 9
3	1, 8
Single-model clusters	3; 10

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 1852 atoms, of which 926 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Protein disulfide-isomerase.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	121	1852	597	926	148	179	2	0

There are 5 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1	GLY	-	EXPRESSION TAG	UNP P55059
A	2	PRO	-	EXPRESSION TAG	UNP P55059
A	3	LEU	-	EXPRESSION TAG	UNP P55059
A	4	GLY	-	EXPRESSION TAG	UNP P55059
A	5	SER	-	EXPRESSION TAG	UNP P55059

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: Protein disulfide-isomerase

Chain A: 47% 36% 8% 9%

G1 P2 L3 G4 S5 E6 G7 P8 V12 V13 A14 K15 N16 Y17 N18 E19 I20 V21 D24 T25 K26 D27 V28 L29 I30 E31 L43 A44 P45 K46 Y47 L50 L53 Y54 A55 K56 S57 E58 V63 V64 I65 A66 K67 V68 D69 N73 D74 V75 I79 Q80 G81 F82

P83 T84 I85 K86 L87 A90 K93 T98 Y99 S102 R103 T104 V105 L108 I109 K110 F111 I112 K119 A120 A121

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

The representative model is number 1. Colouring as in section 4.1 above.

- Molecule 1: Protein disulfide-isomerase

Chain A: 43% 37% 11% 9%

G1 P2 L3 G4 S5 E6 G7 P8 V9 V12 V13 A14 K15 N16 Y17 N18 E19 I20 V21 L22 D23 D24 T25 K26 L29 I30 E31 F32 L43 A44 P45 K46 Y47 E48 E49 L50 L53 Y54 A55 K56 S57 E58 R62 V63 V64 I65 A66 K67 V68 D69 A70 N73 D74 V75

E78 I79 F82 P83 T84 I85 K86 L87 A90 K93 T98 S102 R103 T104 V105 L108 I109 K110 F111 I112 A113 K117 Y118 K119 A120 A121

5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *DGSA-distance geometry simulated annealing.*

Of the 100 calculated structures, 10 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
CNS	refinement	1.3
CYANA	refinement	3.0

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)	2rue_cs.str
Number of chemical shift lists	1
Total number of shifts	1273
Number of shifts mapped to atoms	1273
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	85%

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	857	856	855	36±4
All	All	8570	8560	8550	357

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 21.

5 of 112 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:12:VAL:HG11	1:A:30:ILE:HD12	0.89	1.39	9	7
1:A:24:ASP:O	1:A:90:ALA:HB1	0.76	1.81	1	8
1:A:29:LEU:HD12	1:A:65:ILE:HD13	0.76	1.56	8	6
1:A:79:ILE:HG21	1:A:84:THR:OG1	0.76	1.81	3	10
1:A:85:ILE:HD12	1:A:99:TYR:CD2	0.75	2.17	8	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	110/121 (91%)	98±3 (89±3%)	10±3 (9±3%)	2±1 (2±1%)	18 63
All	All	1100/1210 (91%)	983 (89%)	100 (9%)	17 (2%)	18 63

5 of 6 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	93	LYS	6
1	A	103	ARG	3
1	A	57	SER	3
1	A	76	PRO	2
1	A	77	ASP	2

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	91/97 (94%)	65±3 (71±3%)	26±3 (29±3%)	2 19
All	All	910/970 (94%)	647 (71%)	263 (29%)	2 19

5 of 51 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	43	LEU	10
1	A	54	TYR	10
1	A	93	LYS	10
1	A	82	PHE	10
1	A	18	ASN	10

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 85% for the well-defined parts and 83% for the entire structure.

7.1 Chemical shift list 1

File name: 2rue_cs.str

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	1273
Number of shifts mapped to atoms	1273
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	9

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	115	-0.08 ± 0.18	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	103	0.24 ± 0.16	None needed (< 0.5 ppm)
$^{13}C'$	109	0.16 ± 0.20	None needed (< 0.5 ppm)
^{15}N	103	0.26 ± 0.44	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 85%, i.e. 1125 atoms were assigned a chemical shift out of a possible 1319. 1 out of 19 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	516/538 (96%)	210/214 (98%)	210/220 (95%)	96/104 (92%)
Sidechain	514/669 (77%)	319/388 (82%)	189/258 (73%)	6/23 (26%)

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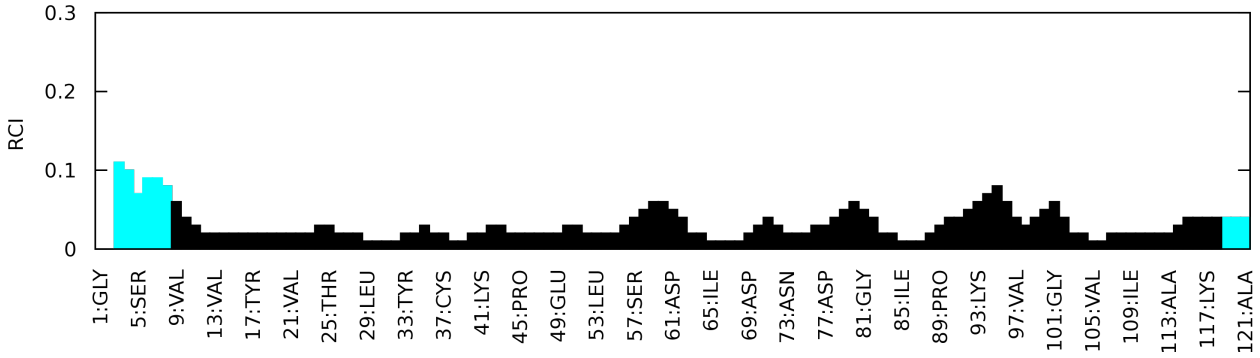
	Total	^{1}H	^{13}C	^{15}N
Aromatic	95/112 (85%)	48/58 (83%)	46/51 (90%)	1/3 (33%)
Overall	1125/1319 (85%)	577/660 (87%)	445/529 (84%)	103/130 (79%)

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	86	LYS	NZ	124.43	49.86 – 18.16	28.5
1	A	35	PRO	CG	51.03	32.66 – 21.76	21.9
1	A	20	ILE	CG1	11.31	36.54 – 18.94	-9.3
1	A	30	ILE	CG2	28.47	24.63 – 10.43	7.7
1	A	34	ALA	HB1	-0.55	2.61 – 0.11	-7.6
1	A	34	ALA	HB2	-0.55	2.61 – 0.11	-7.6
1	A	34	ALA	HB3	-0.55	2.61 – 0.11	-7.6
1	A	20	ILE	CG2	27.83	24.63 – 10.43	7.3
1	A	30	ILE	CG1	15.34	36.54 – 18.94	-7.0

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: