Full wwPDB X-ray Structure Validation Report ⓘ

May 21, 2020 – 10:49 pm BST

PDB ID	:	4GH7
Title	:	Crystal structure of Anticalin N7A in complex with oncofetal fibronectin fragment Fn7B8
Authors	:	Schiefner, A.; Gebauer, M.; Skerra, A.
Deposited on	:	2012-08-07
Resolution	:	2.60 Å(reported)

This is a Full wwPDB X-ray 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/XrayValidationReportHelp
with specific help available everywhere you see the ⓘ symbol.

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

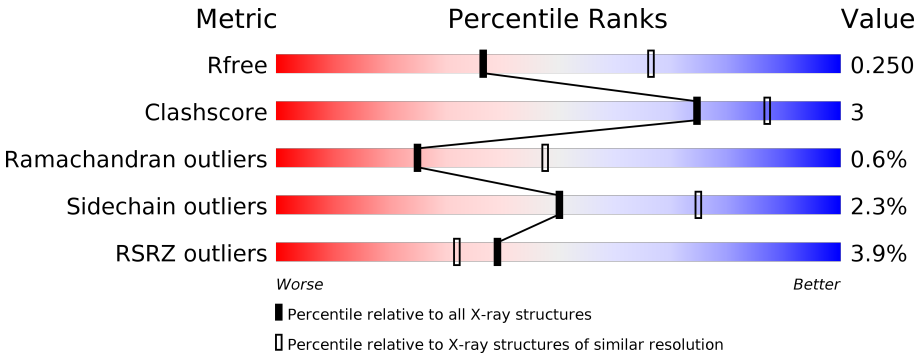
MolProbity	:	4.02b-467
Xtriage (Phenix)	:	1.13
EDS	:	2.11
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.11

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
X-RAY DIFFRACTION

The reported resolution of this entry is 2.60 Å.

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)	Similar resolution (#Entries, resolution range(Å))
R_{free}	130704	3163 (2.60-2.60)
Clashscore	141614	3518 (2.60-2.60)
Ramachandran outliers	138981	3455 (2.60-2.60)
Sidechain outliers	138945	3455 (2.60-2.60)
RSRZ outliers	127900	3104 (2.60-2.60)

The table below summarises the geometric issues observed across the polymeric chains and their fit to the electron density. The red, orange, yellow and green segments on the lower bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria respectively. 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% The upper red bar (where present) indicates the fraction of residues that have poor fit to the electron density. The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	188	3% 82% 11% 7%
1	C	188	4% 78% 14% • 7%
2	B	285	% 89% 7% •
2	D	285	6% 86% 11% ••

2 Entry composition

There are 3 unique types of molecules in this entry. The entry contains 7258 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

- Molecule 1 is a protein called Neutrophil gelatinase-associated lipocalin.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	174	Total	C	N	O	S	0	0	0
			1439	927	251	257	4			
1	C	174	Total	C	N	O	S	0	0	0
			1439	927	251	257	4			

There are 62 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	28	HIS	GLN	ENGINEERED MUTATION	UNP P80188
A	36	LYS	LEU	ENGINEERED MUTATION	UNP P80188
A	40	HIS	ALA	ENGINEERED MUTATION	UNP P80188
A	41	ASP	ILE	ENGINEERED MUTATION	UNP P80188
A	49	ARG	GLN	ENGINEERED MUTATION	UNP P80188
A	52	GLN	TYR	ENGINEERED MUTATION	UNP P80188
A	68	ASN	SER	ENGINEERED MUTATION	UNP P80188
A	70	ARG	LEU	ENGINEERED MUTATION	UNP P80188
A	72	VAL	ARG	ENGINEERED MUTATION	UNP P80188
A	73	HIS	LYS	ENGINEERED MUTATION	UNP P80188
A	77	ASN	ASP	ENGINEERED MUTATION	UNP P80188
A	79	ARG	TRP	ENGINEERED MUTATION	UNP P80188
A	81	TRP	ARG	ENGINEERED MUTATION	UNP P80188
A	87	SER	CYS	ENGINEERED MUTATION	UNP P80188
A	100	TRP	TYR	ENGINEERED MUTATION	UNP P80188
A	106	TRP	TYR	ENGINEERED MUTATION	UNP P80188
A	125	ARG	LYS	ENGINEERED MUTATION	UNP P80188
A	127	TYR	SER	ENGINEERED MUTATION	UNP P80188
A	132	LEU	TYR	ENGINEERED MUTATION	UNP P80188
A	134	GLU	LYS	ENGINEERED MUTATION	UNP P80188
A	146	ASN	SER	ENGINEERED MUTATION	UNP P80188
A	179	SER	-	EXPRESSION TAG	UNP P80188
A	180	ALA	-	EXPRESSION TAG	UNP P80188
A	181	TRP	-	EXPRESSION TAG	UNP P80188
A	182	SER	-	EXPRESSION TAG	UNP P80188

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Chain	Residue	Modelled	Actual	Comment	Reference
A	183	HIS	-	EXPRESSION TAG	UNP P80188
A	184	PRO	-	EXPRESSION TAG	UNP P80188
A	185	GLN	-	EXPRESSION TAG	UNP P80188
A	186	PHE	-	EXPRESSION TAG	UNP P80188
A	187	GLU	-	EXPRESSION TAG	UNP P80188
A	188	LYS	-	EXPRESSION TAG	UNP P80188
C	28	HIS	GLN	ENGINEERED MUTATION	UNP P80188
C	36	LYS	LEU	ENGINEERED MUTATION	UNP P80188
C	40	HIS	ALA	ENGINEERED MUTATION	UNP P80188
C	41	ASP	ILE	ENGINEERED MUTATION	UNP P80188
C	49	ARG	GLN	ENGINEERED MUTATION	UNP P80188
C	52	GLN	TYR	ENGINEERED MUTATION	UNP P80188
C	68	ASN	SER	ENGINEERED MUTATION	UNP P80188
C	70	ARG	LEU	ENGINEERED MUTATION	UNP P80188
C	72	VAL	ARG	ENGINEERED MUTATION	UNP P80188
C	73	HIS	LYS	ENGINEERED MUTATION	UNP P80188
C	77	ASN	ASP	ENGINEERED MUTATION	UNP P80188
C	79	ARG	TRP	ENGINEERED MUTATION	UNP P80188
C	81	TRP	ARG	ENGINEERED MUTATION	UNP P80188
C	87	SER	CYS	ENGINEERED MUTATION	UNP P80188
C	100	TRP	TYR	ENGINEERED MUTATION	UNP P80188
C	106	TRP	TYR	ENGINEERED MUTATION	UNP P80188
C	125	ARG	LYS	ENGINEERED MUTATION	UNP P80188
C	127	TYR	SER	ENGINEERED MUTATION	UNP P80188
C	132	LEU	TYR	ENGINEERED MUTATION	UNP P80188
C	134	GLU	LYS	ENGINEERED MUTATION	UNP P80188
C	146	ASN	SER	ENGINEERED MUTATION	UNP P80188
C	179	SER	-	EXPRESSION TAG	UNP P80188
C	180	ALA	-	EXPRESSION TAG	UNP P80188
C	181	TRP	-	EXPRESSION TAG	UNP P80188
C	182	SER	-	EXPRESSION TAG	UNP P80188
C	183	HIS	-	EXPRESSION TAG	UNP P80188
C	184	PRO	-	EXPRESSION TAG	UNP P80188
C	185	GLN	-	EXPRESSION TAG	UNP P80188
C	186	PHE	-	EXPRESSION TAG	UNP P80188
C	187	GLU	-	EXPRESSION TAG	UNP P80188
C	188	LYS	-	EXPRESSION TAG	UNP P80188

- Molecule 2 is a protein called Fibronectin.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	B	275	Total	C	N	O	S	0	0	0
			2099	1321	336	440	2			

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Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	D	278	Total	C	N	O	S	0	0	0
			2117	1330	341	444	2			

There are 18 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	1172	MET	-	EXPRESSION TAG	UNP P02751
B	1449	SER	-	EXPRESSION TAG	UNP P02751
B	1450	ALA	-	EXPRESSION TAG	UNP P02751
B	1451	HIS	-	EXPRESSION TAG	UNP P02751
B	1452	HIS	-	EXPRESSION TAG	UNP P02751
B	1453	HIS	-	EXPRESSION TAG	UNP P02751
B	1454	HIS	-	EXPRESSION TAG	UNP P02751
B	1455	HIS	-	EXPRESSION TAG	UNP P02751
B	1456	HIS	-	EXPRESSION TAG	UNP P02751
D	1172	MET	-	EXPRESSION TAG	UNP P02751
D	1449	SER	-	EXPRESSION TAG	UNP P02751
D	1450	ALA	-	EXPRESSION TAG	UNP P02751
D	1451	HIS	-	EXPRESSION TAG	UNP P02751
D	1452	HIS	-	EXPRESSION TAG	UNP P02751
D	1453	HIS	-	EXPRESSION TAG	UNP P02751
D	1454	HIS	-	EXPRESSION TAG	UNP P02751
D	1455	HIS	-	EXPRESSION TAG	UNP P02751
D	1456	HIS	-	EXPRESSION TAG	UNP P02751

- Molecule 3 is water.

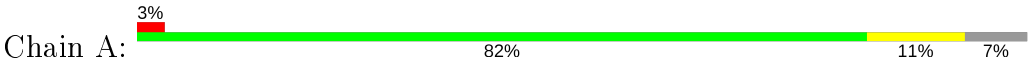
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
3	A	27	Total	O	0	0
			27	27		
3	B	63	Total	O	0	0
			63	63		
3	C	12	Total	O	0	0
			12	12		
3	D	62	Total	O	0	0
			62	62		

3 Residue-property plots (i)

These plots are drawn for all protein, RNA and DNA chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and electron density. Residues are color-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. A red dot above a residue indicates a poor fit to the electron density (RSRZ > 2). Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

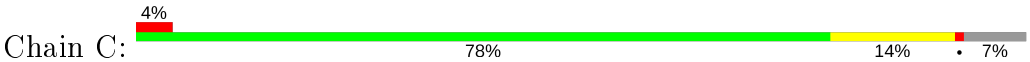
- Molecule 1: Neutrophil gelatinase-associated lipocalin

Chain A:



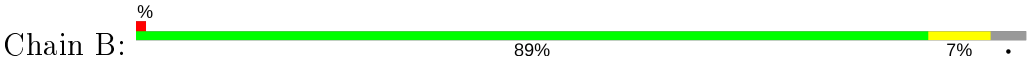
- Molecule 1: Neutrophil gelatinase-associated lipocalin

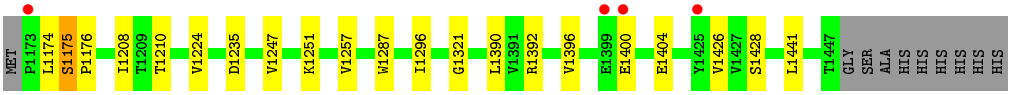
Chain C:



- Molecule 2: Fibronectin

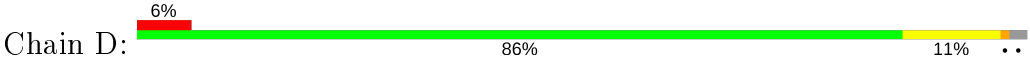
Chain B:

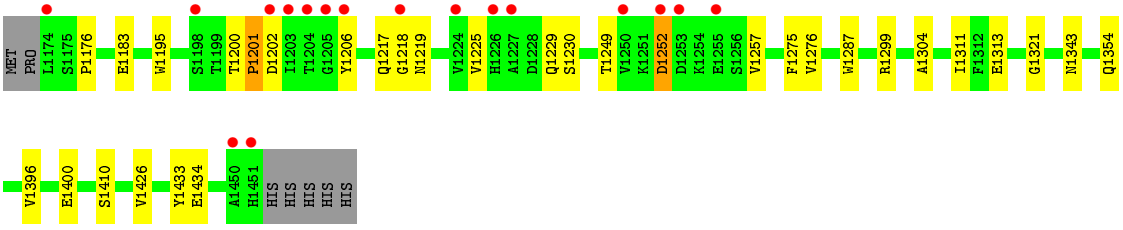




- Molecule 2: Fibronectin

Chain D:





4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 43 21 2	Depositor
Cell constants a, b, c, α, β, γ	107.13Å 107.13Å 233.16Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	30.00 – 2.60 29.47 – 2.60	Depositor EDS
% Data completeness (in resolution range)	99.8 (30.00-2.60) 99.8 (29.47-2.60)	Depositor EDS
R_{merge}	0.08	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	2.87 (at 2.61Å)	Xtriage
Refinement program	REFMAC	Depositor
R, R_{free}	0.218 , 0.259 0.212 , 0.250	Depositor DCC
R_{free} test set	2148 reflections (5.05%)	wwPDB-VP
Wilson B-factor (Å^2)	51.0	Xtriage
Anisotropy	0.001	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.31 , 33.4	EDS
L-test for twinning[2]	$< \|L\| > = 0.48$, $< L^2 > = 0.31$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.93	EDS
Total number of atoms	7258	wwPDB-VP
Average B, all atoms (Å^2)	57.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 2.52% of the height of the origin peak. No significant pseudotranslation is detected.*

[1] Intensities estimated from amplitudes.

[2] Theoretical values of $< |L| >$, $< L^2 >$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

5 Model quality

5.1 Standard geometry

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 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	0.43	0/1480	0.55	0/2006
1	C	0.41	0/1480	0.53	0/2006
2	B	0.39	0/2148	0.56	0/2959
2	D	0.39	0/2166	0.55	0/2983
All	All	0.40	0/7274	0.55	0/9954

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no planarity outliers.

5.2 Too-close contacts

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in the 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 within the asymmetric unit, whereas Symm-Clashes lists symmetry related clashes.

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	1439	0	1417	9	0
1	C	1439	0	1417	13	0
2	B	2099	0	2041	9	0
2	D	2117	0	2053	16	0
3	A	27	0	0	0	0
3	B	63	0	0	0	0
3	C	12	0	0	0	0
3	D	62	0	0	0	0
All	All	7258	0	6928	45	0

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 3.

All (45) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:C:45:ASP:O	1:C:46:LYS:HB2	1.83	0.78
2:B:1174:LEU:HD11	2:B:1251:LYS:HB3	1.67	0.75
1:C:145:THR:HB	1:C:148:LEU:HD13	1.71	0.73
1:A:49:ARG:HD2	1:A:167:VAL:HG11	1.71	0.72
1:A:91:GLU:OE2	1:C:87:SER:HB3	1.92	0.70
1:C:145:THR:HG22	1:C:147:GLU:H	1.57	0.69
1:A:11:PRO:HG3	1:A:159:LEU:HG	1.74	0.69
2:D:1396:VAL:HG22	2:D:1426:VAL:CG2	2.23	0.67
2:D:1396:VAL:HG22	2:D:1426:VAL:HG22	1.77	0.67
1:C:11:PRO:HG3	1:C:159:LEU:HG	1.79	0.64
1:C:114:ASN:HD21	1:C:117:GLN:HB2	1.70	0.56
2:D:1200:THR:HB	2:D:1201:PRO:HD2	1.88	0.55
1:A:45:ASP:O	1:A:46:LYS:HB2	2.08	0.53
2:D:1304:ALA:HB2	2:D:1311:ILE:HD11	1.91	0.52
2:D:1195:TRP:CE2	2:D:1230:SER:HA	2.45	0.52
2:B:1296:ILE:O	2:D:1343:ASN:ND2	2.39	0.51
1:A:145:THR:HG22	1:A:147:GLU:H	1.76	0.49
2:D:1206:TYR:CE1	2:D:1249:THR:HG22	2.48	0.49
1:C:49:ARG:HD2	1:C:167:VAL:HG11	1.95	0.49
1:A:17:PRO:HG2	1:A:111:VAL:HG13	1.95	0.48
2:D:1225:VAL:HB	2:D:1229:GLN:OE1	2.13	0.48
1:C:163:GLU:HA	1:C:166:ILE:HD12	1.97	0.46
1:A:114:ASN:HD21	1:A:117:GLN:HB2	1.80	0.45
2:D:1176:PRO:HB3	2:D:1257:VAL:O	2.16	0.45
1:C:88:GLN:HG2	1:C:91:GLU:OE1	2.16	0.45
2:B:1392:ARG:HG2	2:B:1404:GLU:HG3	1.99	0.45
2:D:1201:PRO:HB2	2:D:1202:ASP:H	1.57	0.45
1:A:84:VAL:HG13	1:A:85:PRO:HD2	1.99	0.45
1:C:64:TYR:HE1	1:C:92:PHE:CZ	2.36	0.44
2:B:1176:PRO:HB3	2:B:1257:VAL:O	2.18	0.44
2:B:1287:TRP:CE2	2:B:1321:GLY:HA2	2.53	0.44
1:A:161:LEU:HA	1:A:162:PRO:HD3	1.89	0.43
2:D:1275:PHE:CE2	2:D:1354:GLN:HB2	2.53	0.43
1:C:91:GLU:OE2	1:C:109:ARG:NH2	2.49	0.43
2:D:1200:THR:CB	2:D:1201:PRO:HD2	2.49	0.42
2:D:1217:GLN:O	2:D:1219:ASN:N	2.51	0.42
2:D:1433:TYR:CE1	2:D:1434:GLU:HG2	2.54	0.42
2:B:1208:ILE:HG13	2:B:1247:VAL:HG13	2.01	0.42
2:B:1175:SER:HA	2:B:1176:PRO:HD2	1.84	0.41
2:B:1396:VAL:HG22	2:B:1426:VAL:HG23	2.02	0.41

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:C:66:VAL:HG21	1:C:83:PHE:CD1	2.55	0.41
2:D:1299:ARG:HD2	2:D:1313:GLU:OE1	2.20	0.41
1:C:102:GLY:O	1:C:127:TYR:HA	2.21	0.41
2:D:1287:TRP:CE2	2:D:1321:GLY:HA2	2.56	0.41
2:B:1428:SER:HA	2:B:1441:LEU:O	2.21	0.40

There are no symmetry-related clashes.

5.3 Torsion angles (i)

5.3.1 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 X-ray entries followed by that with respect to entries of similar resolution.

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	172/188 (92%)	165 (96%)	7 (4%)	0	100	100
1	C	172/188 (92%)	164 (95%)	6 (4%)	2 (1%)	13	27
2	B	273/285 (96%)	265 (97%)	8 (3%)	0	100	100
2	D	276/285 (97%)	263 (95%)	10 (4%)	3 (1%)	14	30
All	All	893/946 (94%)	857 (96%)	31 (4%)	5 (1%)	25	47

All (5) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	C	46	LYS
2	D	1201	PRO
2	D	1218	GLY
2	D	1252	ASP
1	C	6	ASP

5.3.2 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 X-ray entries followed by that with respect to entries of similar

resolution.

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	160/173 (92%)	157 (98%)	3 (2%)	57	79
1	C	160/173 (92%)	155 (97%)	5 (3%)	40	66
2	B	249/257 (97%)	243 (98%)	6 (2%)	49	74
2	D	250/257 (97%)	245 (98%)	5 (2%)	55	78
All	All	819/860 (95%)	800 (98%)	19 (2%)	50	75

All (19) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	7	LEU
1	A	14	SER
1	A	21	ASN
2	B	1175	SER
2	B	1210	THR
2	B	1224	VAL
2	B	1235	ASP
2	B	1390	LEU
2	B	1400	GLU
1	C	30	LYS
1	C	42	LEU
1	C	46	LYS
1	C	60	GLU
1	C	72	VAL
2	D	1183	GLU
2	D	1252	ASP
2	D	1276	VAL
2	D	1400	GLU
2	D	1410	SER

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

5.3.3 RNA

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains

There are no non-standard protein/DNA/RNA residues in this entry.

5.5 Carbohydrates

There are no carbohydrates in this entry.

5.6 Ligand geometry

There are no ligands in this entry.

5.7 Other polymers

There are no such residues in this entry.

5.8 Polymer linkage issues

There are no chain breaks in this entry.

6 Fit of model and data

6.1 Protein, DNA and RNA chains

In the following table, the column labelled '#RSRZ> 2' contains the number (and percentage) of RSRZ outliers, followed by percent RSRZ outliers for the chain as percentile scores relative to all X-ray entries and entries of similar resolution. The OWAB column contains the minimum, median, 95^{th} percentile and maximum values of the occupancy-weighted average B-factor per residue. The column labelled 'Q< 0.9' lists the number of (and percentage) of residues with an average occupancy less than 0.9.

Mol	Chain	Analysed	<RSRZ>	#RSRZ>2			OWAB(Å^2)	Q<0.9
1	A	174/188 (92%)	-0.11	6 (3%)	45	38	27, 50, 101, 154	0
1	C	174/188 (92%)	0.02	8 (4%)	32	26	38, 57, 99, 150	0
2	B	275/285 (96%)	-0.39	4 (1%)	73	70	24, 45, 104, 136	0
2	D	278/285 (97%)	-0.11	17 (6%)	21	16	24, 47, 147, 204	0
All	All	901/946 (95%)	-0.17	35 (3%)	39	32	24, 51, 116, 204	0

All (35) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	C	46	LYS	7.0
1	C	45	ASP	6.9
2	D	1203	ILE	6.3
1	C	44	GLU	6.2
2	D	1218	GLY	4.0
2	D	1204	THR	3.7
1	C	43	ARG	3.7
2	B	1399	GLU	3.4
1	A	46	LYS	3.4
2	D	1227	ALA	3.3
1	C	178	GLY	3.3
2	D	1255	GLU	3.2
2	D	1250	VAL	3.2
2	D	1206	TYR	3.2
1	A	45	ASP	3.1
1	C	6	ASP	3.0
2	D	1451	HIS	2.9
1	A	5	SER	2.9
2	D	1174	LEU	2.9
1	C	47	ASP	2.8
2	D	1202	ASP	2.7

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Mol	Chain	Res	Type	RSRZ
1	C	5	SER	2.7
2	B	1425	TYR	2.6
1	A	43	ARG	2.5
2	D	1253	ASP	2.5
2	D	1252	ASP	2.4
2	D	1450	ALA	2.3
2	D	1224	VAL	2.3
2	D	1226	HIS	2.3
2	D	1205	GLY	2.2
1	A	42	LEU	2.1
1	A	47	ASP	2.1
2	B	1173	PRO	2.0
2	D	1198	SER	2.0
2	B	1400	GLU	2.0

6.2 Non-standard residues in protein, DNA, RNA chains (i)

There are no non-standard protein/DNA/RNA residues in this entry.

6.3 Carbohydrates (i)

There are no carbohydrates in this entry.

6.4 Ligands (i)

There are no ligands in this entry.

6.5 Other polymers (i)

There are no such residues in this entry.