wwPDB X-ray Structure Validation Summary Report (i)

Aug 20, 2023 – 10:09 AM EDT

PDB ID	:	2NZ9
Title	:	Crystal structure of botulinum neurotoxin type A complexed with monoclonal antibody AR2
Authors	:	Stevens, R.C.; Arndt, J.W.
Deposited on	:	2006-11-22
Resolution	:	3.79 Å(reported)

This is a wwPDB X-ray 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/XrayValidationReportHelp
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 (1)) were used in the production of this report:

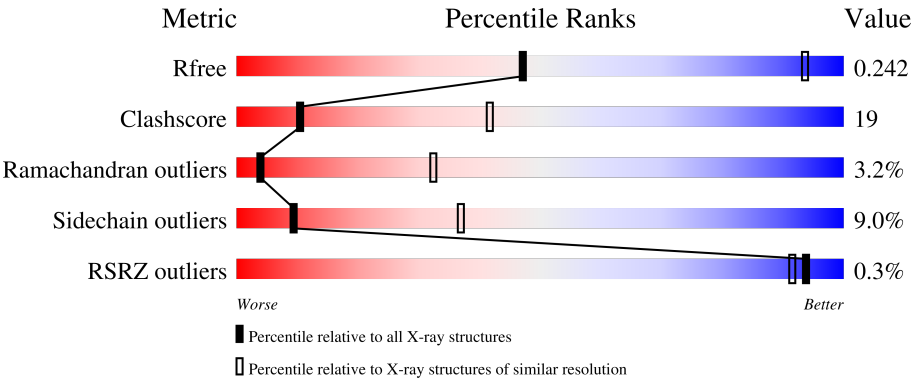
MolProbity	:	4.02b-467
Xtriage (Phenix)	:	1.13
EDS	:	2.35
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.35

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 3.79 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.



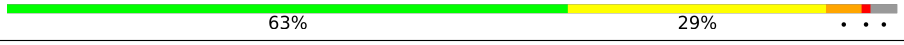
Metric	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
R_{free}	130704	1212 (4.00-3.60)
Clashscore	141614	1288 (4.00-3.60)
Ramachandran outliers	138981	1243 (4.00-3.60)
Sidechain outliers	138945	1237 (4.00-3.60)
RSRZ outliers	127900	1121 (4.00-3.60)

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 of 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	1295	61% 31% 5%
1	B	1295	% 63% 30% 5%
2	C	218	60% 33% 6%
2	E	218	62% 30% 6%
3	D	224	63% 27% 5%

Continued on next page...

Continued from previous page...

Mol	Chain	Length	Quality of chain
3	F	224	

2 Entry composition (i)

There are 5 unique types of molecules in this entry. The entry contains 26985 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 Botulinum neurotoxin type A.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	1267	Total	C	N	O	S	0	0	0
			10200	6541	1691	1937	31			
1	B	1267	Total	C	N	O	S	0	0	0
			10203	6543	1691	1938	31			

- Molecule 2 is a protein called AR2 monoclonal antibody.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	C	216	Total	C	N	O	S	0	0	0
			1657	1039	279	334	5			
2	E	216	Total	C	N	O	S	0	0	0
			1657	1039	279	334	5			

- Molecule 3 is a protein called AR2 monoclonal antibody.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
3	D	217	Total	C	N	O	S	0	0	0
			1632	1025	271	329	7			
3	F	217	Total	C	N	O	S	0	0	0
			1632	1025	271	329	7			

- Molecule 4 is ZINC ION (three-letter code: ZN) (formula: Zn).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
4	A	1	Total	Zn	0	0
			1	1		
4	B	1	Total	Zn	0	0
			1	1		

- Molecule 5 is CALCIUM ION (three-letter code: CA) (formula: Ca).

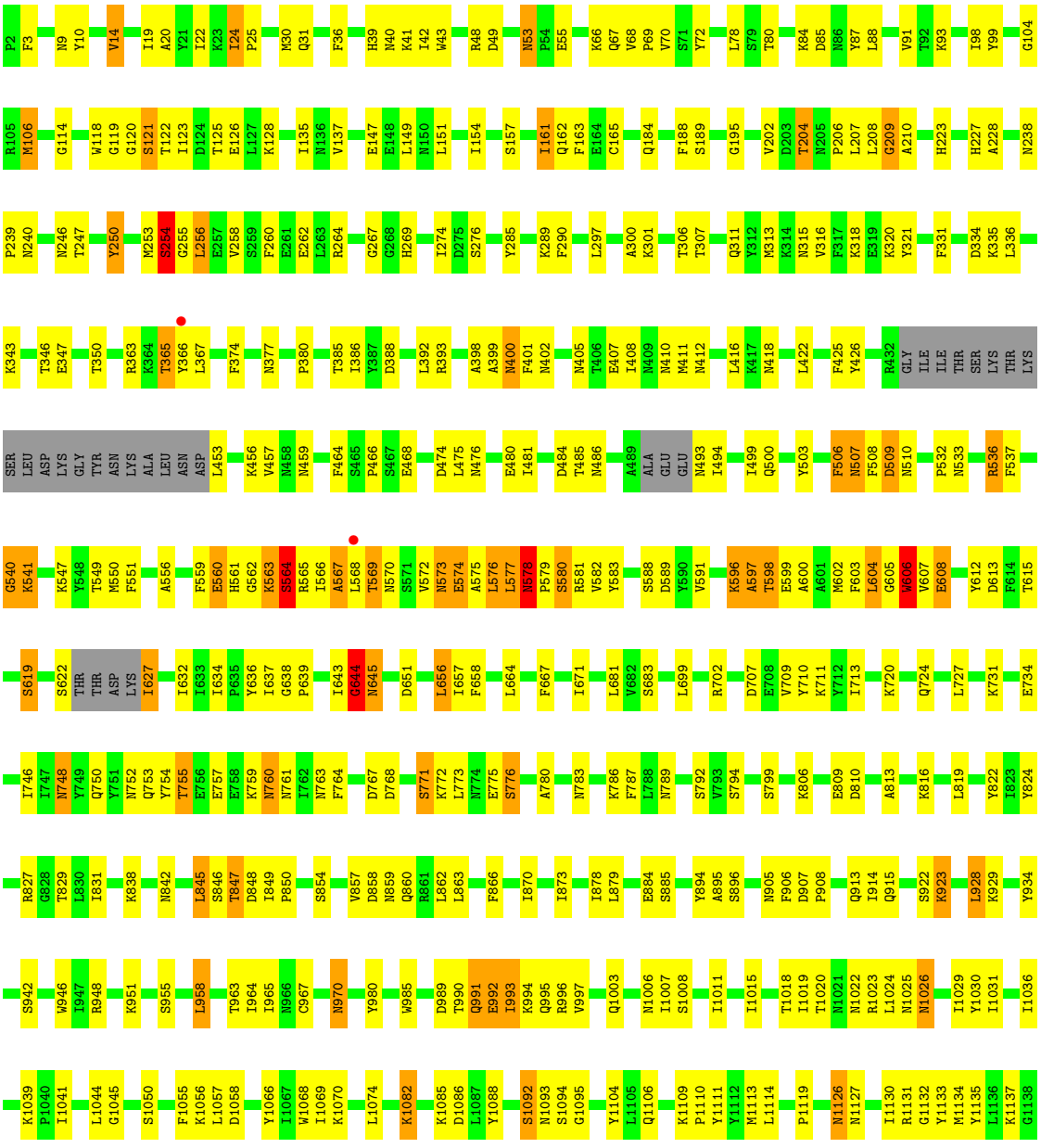
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	A	1	Total Ca 1 1	0	0
5	B	1	Total Ca 1 1	0	0

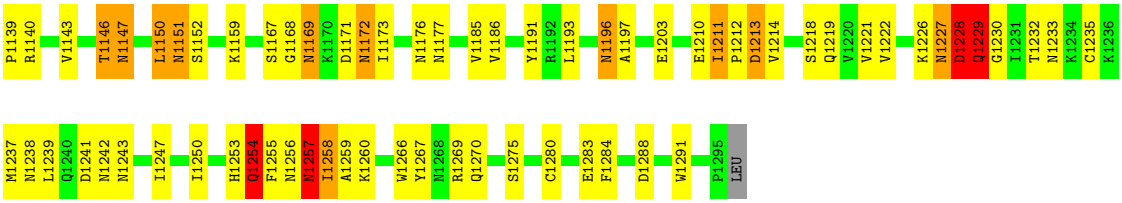
3 Residue-property plots (i)

These plots are drawn for all protein, RNA, DNA and oligosaccharide 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: Botulinum neurotoxin type A

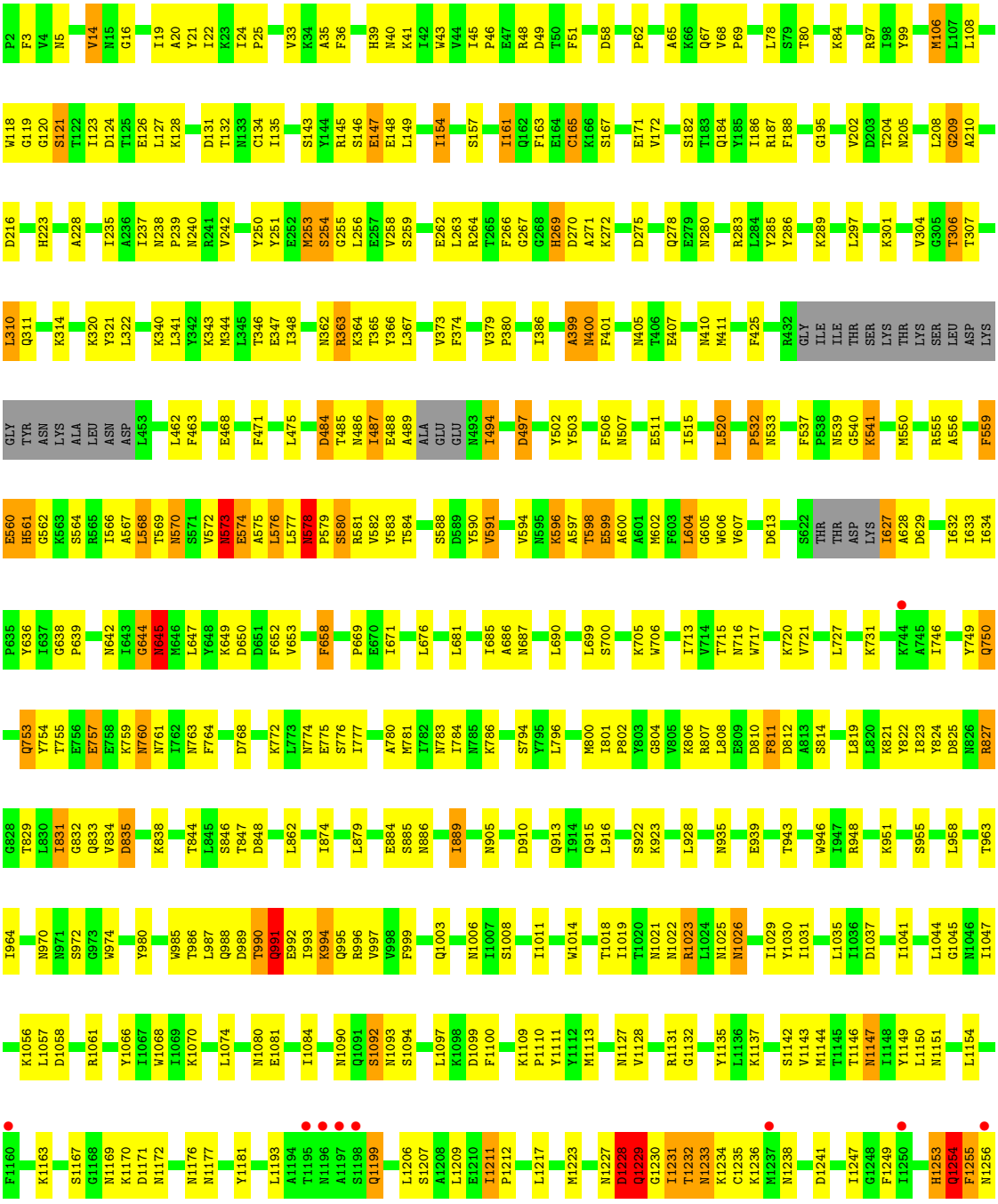
Chain A:





• Molecule 1: Botulinum neurotoxin type A

Chain B:

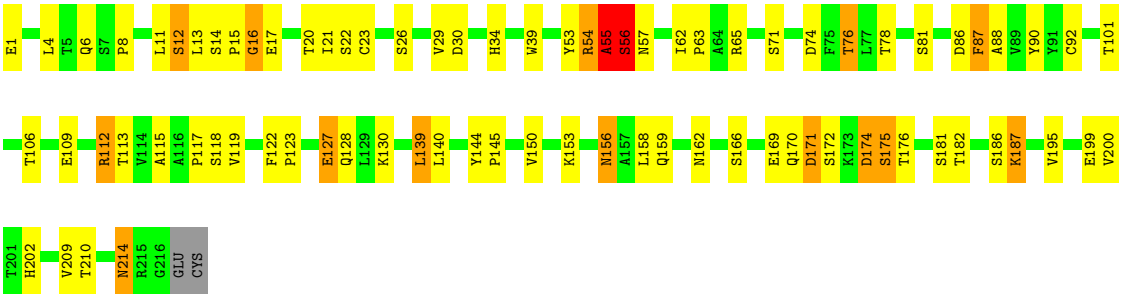


• Molecule 2: AR2 monoclonal antibody

Chain C:

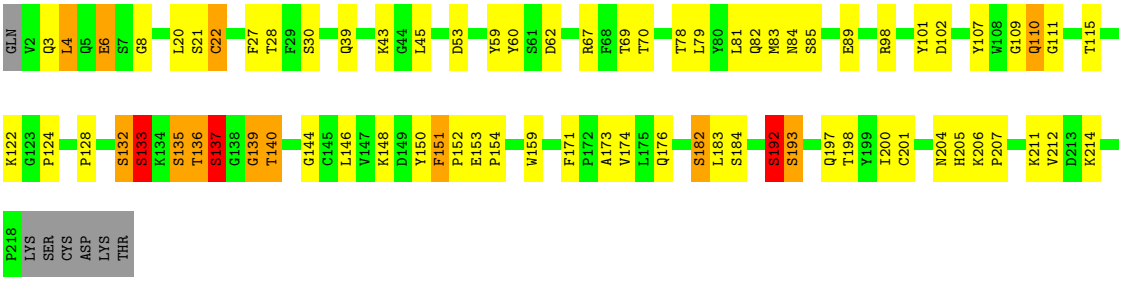
• Molecule 2: AR2 monoclonal antibody

Chain E:



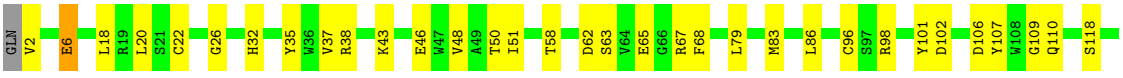
• Molecule 3: AR2 monoclonal antibody

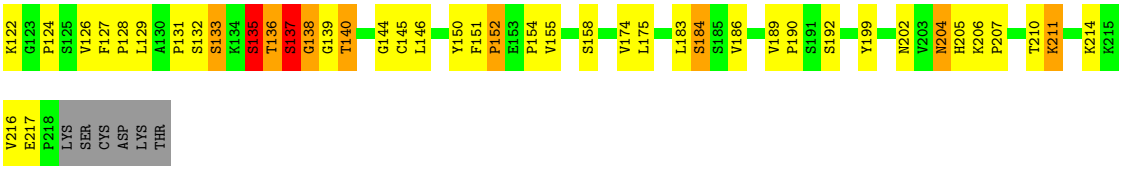
Chain D:



• Molecule 3: AR2 monoclonal antibody

Chain F:




K122
G123
P124
S125
V126
F127
P128
L129
A130
P131
S132
S133
K134
S135
T136
S137
G138
G139
T140
G144
C145
L146
Y150
F151
P152
E153
P154
V155
S158
V174
L175
L183
S184
S185
V186
V189
P190
S191
S192
Y199
N202
V203
N204
H205
K206
P207
T210
K211
K214
K215
V216
E217
P218
LYS
SER
CYS
ASP
LYS
THR

4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α, β, γ	97.97Å 197.60Å 146.10Å 90.00° 91.18° 90.00°	Depositor
Resolution (Å)	40.00 – 3.79 48.97 – 3.79	Depositor EDS
% Data completeness (in resolution range)	95.5 (40.00-3.79) 95.5 (48.97-3.79)	Depositor EDS
R_{merge}	0.13	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	2.90 (at 3.77Å)	Xtriage
Refinement program	REFMAC 5.2.0019	Depositor
R, R_{free}	0.224 , 0.278 0.203 , 0.242	Depositor DCC
R_{free} test set	2688 reflections (5.11%)	wwPDB-VP
Wilson B-factor (Å^2)	95.2	Xtriage
Anisotropy	0.122	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.29 , 112.2	EDS
L-test for twinning[2]	$< \|L\| > = 0.45$, $< L^2 > = 0.27$	Xtriage
Estimated twinning fraction	0.085 for h,-k,-l	Xtriage
F_o,F_c correlation	0.92	EDS
Total number of atoms	26985	wwPDB-VP
Average B, all atoms (Å^2)	84.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 2.34% 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

Bond lengths and bond angles in the following residue types are not validated in this section: CA, ZN

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	0.64	0/10416	0.67	0/14098
1	B	0.64	0/10420	0.66	0/14104
2	C	0.64	0/1695	0.60	0/2303
2	E	0.65	1/1695 (0.1%)	0.62	0/2303
3	D	0.56	0/1673	0.66	1/2281 (0.0%)
3	F	0.52	0/1673	0.64	0/2281
All	All	0.63	1/27572 (0.0%)	0.65	1/37370 (0.0%)

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand.A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	#Chirality outliers	#Planarity outliers
1	A	0	26
1	B	0	27
2	C	0	3
2	E	0	3
3	D	0	6
3	F	0	5
All	All	0	70

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	E	127	GLU	CD-OE1	5.77	1.31	1.25

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	D	153	GLU	C-N-CD	-8.33	102.27	120.60

There are no chirality outliers.

5 of 70 planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	118	TRP	Peptide
1	A	119	GLY	Peptide
1	A	484	ASP	Peptide
1	A	507	ASN	Peptide
1	A	547	LYS	Peptide

5.2 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 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	10200	0	10003	357	0
1	B	10203	0	9999	372	0
2	C	1657	0	1595	64	0
2	E	1657	0	1595	70	0
3	D	1632	0	1552	64	0
3	F	1632	0	1552	73	0
4	A	1	0	0	0	0
4	B	1	0	0	0	0
5	A	1	0	0	0	0
5	B	1	0	0	0	0
All	All	26985	0	26296	992	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 19.

The worst 5 of 992 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:B:269:HIS:CD2	1:B:365:THR:HG21	1.40	1.56
1:A:596:LYS:CB	1:A:597:ALA:HB3	1.39	1.47

Continued on next page...

Continued from previous page...

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
3:F:132:SER:HA	3:F:133:SER:CB	1.42	1.46
1:B:540:GLY:CA	1:B:541:LYS:HB2	1.40	1.43
1:B:578:ASN:HB3	1:B:579:PRO:CA	1.41	1.43

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	1259/1295 (97%)	1084 (86%)	130 (10%)	45 (4%)	3 30
1	B	1259/1295 (97%)	1081 (86%)	139 (11%)	39 (3%)	4 33
2	C	214/218 (98%)	182 (85%)	30 (14%)	2 (1%)	17 54
2	E	214/218 (98%)	193 (90%)	14 (6%)	7 (3%)	4 32
3	D	215/224 (96%)	182 (85%)	24 (11%)	9 (4%)	3 26
3	F	215/224 (96%)	192 (89%)	17 (8%)	6 (3%)	5 35
All	All	3376/3474 (97%)	2914 (86%)	354 (10%)	108 (3%)	4 32

5 of 108 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	121	SER
1	A	400	ASN
1	A	560	GLU
1	A	564	SER
1	A	566	ILE

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	1124/1178 (95%)	1020 (91%)	104 (9%)	9 35
1	B	1124/1178 (95%)	1037 (92%)	87 (8%)	13 43
2	C	186/190 (98%)	165 (89%)	21 (11%)	6 28
2	E	186/190 (98%)	165 (89%)	21 (11%)	6 28
3	D	181/190 (95%)	162 (90%)	19 (10%)	7 30
3	F	181/190 (95%)	165 (91%)	16 (9%)	10 38
All	All	2982/3116 (96%)	2714 (91%)	268 (9%)	9 37

5 of 268 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	E	1	GLU
2	E	76	THR
3	F	118	SER
1	A	1218	SER
1	A	1196	ASN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 48 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	383	ASN
1	B	1233	ASN
1	B	833	GLN
1	B	1172	ASN
1	B	1256	ASN

5.3.3 RNA (i)

There are no RNA molecules in this entry.

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

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

5.5 Carbohydrates (i)

There are no monosaccharides in this entry.

5.6 Ligand geometry (i)

Of 4 ligands modelled in this entry, 4 are monoatomic - leaving 0 for Mogul analysis.

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

No monomer is involved in short contacts.

5.7 Other polymers (i)

There are no such residues in this entry.

5.8 Polymer linkage issues (i)

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	1267/1295 (97%)	-0.17	2 (0%) 95 94	29, 84, 98, 110	0
1	B	1267/1295 (97%)	-0.15	9 (0%) 87 83	33, 85, 97, 111	0
2	C	216/218 (99%)	-0.14	0 100 100	62, 84, 92, 95	0
2	E	216/218 (99%)	-0.26	0 100 100	67, 85, 92, 95	0
3	D	217/224 (96%)	-0.15	0 100 100	75, 85, 95, 103	0
3	F	217/224 (96%)	-0.18	0 100 100	72, 84, 92, 99	0
All	All	3400/3474 (97%)	-0.17	11 (0%) 94 91	29, 84, 97, 111	0

The worst 5 of 11 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	366	TYR	3.2
1	B	1198	SER	2.8
1	B	1237	MET	2.4
1	B	1160	PHE	2.3
1	B	1256	ASN	2.2

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

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

6.3 Carbohydrates

There are no monosaccharides in this entry.

6.4 Ligands

In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95^{th} percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists the number of atoms with occupancy less than 0.9.

Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å^2)	Q<0.9
5	CA	A	1297	1/1	0.85	0.23	101,101,101,101	0
5	CA	B	1298	1/1	0.91	0.14	110,110,110,110	0
4	ZN	A	1	1/1	0.96	0.15	81,81,81,81	0
4	ZN	B	1297	1/1	0.99	0.13	72,72,72,72	0

6.5 Other polymers

There are no such residues in this entry.