



# wwPDB X-ray Structure Validation Summary Report ⓘ

Aug 21, 2020 – 03:27 PM BST

PDB ID : 4KUF  
Title : Crystal structure of the catalytic domain of botulinum neurotoxin BoNT/A C134 mutant with MTSEA modified Cys-165 causing stretch disorder  
Authors : Stura, E.A.; Vera, L.; Ptchelkine, D.; Bakirci, H.; Garcia, S.; Dive, V.  
Deposited on : 2013-05-22  
Resolution : 1.70 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](mailto: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.

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

MolProbity : 4.02b-467  
Mogul : 1.8.5 (274361), CSD as541be (2020)  
Xtriage (Phenix) : 1.13  
EDS : 2.13.1  
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) : 2.13.1

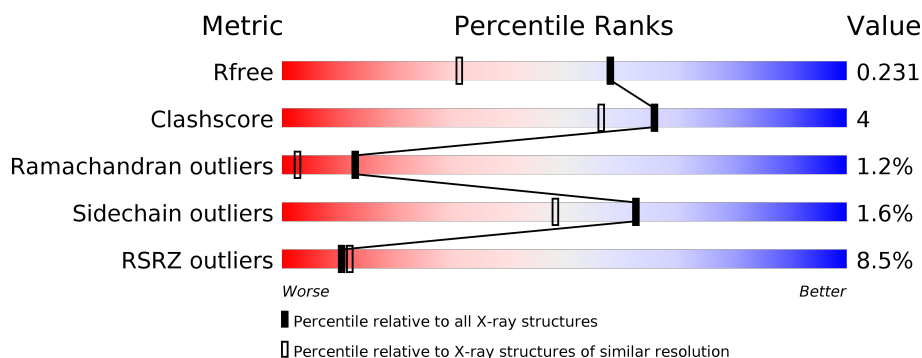
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

## *X-RAY DIFFRACTION*

The reported resolution of this entry is 1.70 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	4298 (1.70-1.70)
Clashscore	141614	4695 (1.70-1.70)
Ramachandran outliers	138981	4610 (1.70-1.70)
Sidechain outliers	138945	4610 (1.70-1.70)
RSRZ outliers	127900	4222 (1.70-1.70)

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  $\geq 3$ , 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  $\leq 5\%$ . 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	445	<div> <div>8%</div> <div>85%</div> <div>9%</div> <div>• •</div> </div>

## 2 Entry composition

There are 8 unique types of molecules in this entry. The entry contains 3941 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 A light chain.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	426	3539	2285	578	666	10	0	13	0

There are 21 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-19	MET	-	EXPRESSION TAG	UNP A5HZZ9
A	-18	GLY	-	EXPRESSION TAG	UNP A5HZZ9
A	-17	SER	-	EXPRESSION TAG	UNP A5HZZ9
A	-16	SER	-	EXPRESSION TAG	UNP A5HZZ9
A	-15	HIS	-	EXPRESSION TAG	UNP A5HZZ9
A	-14	HIS	-	EXPRESSION TAG	UNP A5HZZ9
A	-13	HIS	-	EXPRESSION TAG	UNP A5HZZ9
A	-12	HIS	-	EXPRESSION TAG	UNP A5HZZ9
A	-11	HIS	-	EXPRESSION TAG	UNP A5HZZ9
A	-10	HIS	-	EXPRESSION TAG	UNP A5HZZ9
A	-9	SER	-	EXPRESSION TAG	UNP A5HZZ9
A	-8	SER	-	EXPRESSION TAG	UNP A5HZZ9
A	-7	GLY	-	EXPRESSION TAG	UNP A5HZZ9
A	-6	LEU	-	EXPRESSION TAG	UNP A5HZZ9
A	-5	VAL	-	EXPRESSION TAG	UNP A5HZZ9
A	-4	PRO	-	EXPRESSION TAG	UNP A5HZZ9
A	-3	ARG	-	EXPRESSION TAG	UNP A5HZZ9
A	-2	GLY	-	EXPRESSION TAG	UNP A5HZZ9
A	-1	SER	-	EXPRESSION TAG	UNP A5HZZ9
A	0	HIS	-	EXPRESSION TAG	UNP A5HZZ9
A	134	SER	CYS	ENGINEERED MUTATION	UNP A5HZZ9

- Molecule 2 is ZINC ION (three-letter code: ZN) (formula: Zn).

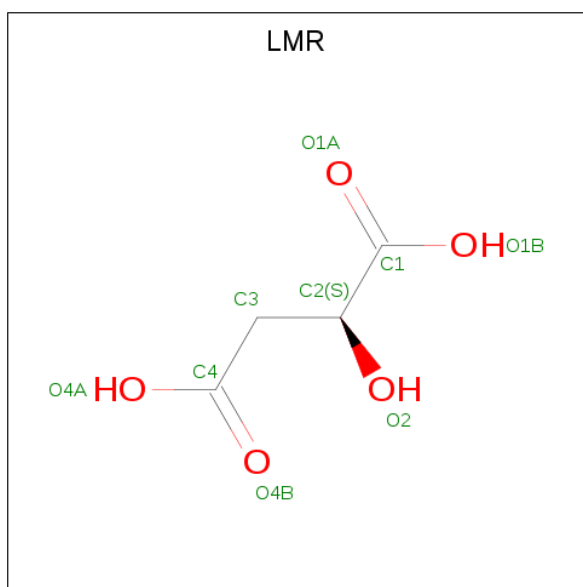
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
2	A	1	Total	Zn	0	0
			1	1		

- Molecule 3 is DIMETHYL SULFOXIDE (three-letter code: DMS) (formula:  $C_2H_6OS$ ).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
3	A	1	Total	C	O	S	0	0
			4	2	1	1		
3	A	1	Total	C	O	S	0	0
			4	2	1	1		
3	A	1	Total	C	O	S	0	0
			4	2	1	1		
3	A	1	Total	C	O	S	0	0
			4	2	1	1		

- Molecule 4 is (2S)-2-hydroxybutanedioic acid (three-letter code: LMR) (formula:  $C_4H_6O_5$ ).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
4	A	1	Total	C	O	0	0
			9	4	5		

- Molecule 5 is GLYCEROL (three-letter code: GOL) (formula:  $C_3H_8O_3$ ).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
5	A	1	Total	C	O	0	0
			6	3	3		

- Molecule 6 is 1,2-ETHANEDIOL (three-letter code: EDO) (formula:  $C_2H_6O_2$ ).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
6	A	1	Total	C	O	0	0
			4	2	2		
6	A	1	Total	C	O	0	0
			4	2	2		
6	A	1	Total	C	O	0	0
			4	2	2		
6	A	1	Total	C	O	0	0
			4	2	2		
6	A	1	Total	C	O	0	0
			4	2	2		
6	A	1	Total	C	O	0	0
			4	2	2		
6	A	1	Total	C	O	0	0
			4	2	2		

- Molecule 7 is SODIUM ION (three-letter code: NA) (formula: Na).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
7	A	2	Total	Na	0	0
			2	2		

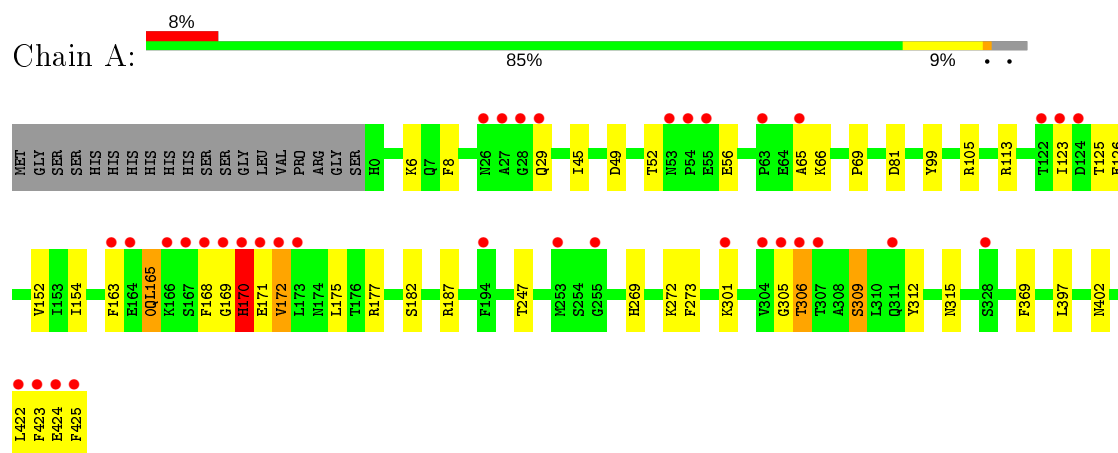
- Molecule 8 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
8	A	332	Total 332	O 332	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 A light chain





## 4 Data and refinement statistics

Property	Value	Source
Space group	P 43 21 2	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	65.46 Å 65.46 Å 201.62 Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	39.94 – 1.70 39.94 – 1.70	Depositor EDS
% Data completeness (in resolution range)	99.7 (39.94-1.70) 99.7 (39.94-1.70)	Depositor EDS
$R_{merge}$	0.15	Depositor
$R_{sym}$	0.14	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	1.43 (at 1.70 Å)	Xtriage
Refinement program	PHENIX (phenix.refine: 1.8 _1069), REFMAC	Depositor
R, $R_{free}$	0.180 , 0.231 0.182 , 0.231	Depositor DCC
$R_{free}$ test set	2475 reflections (5.00%)	wwPDB-VP
Wilson B-factor (Å <sup>2</sup> )	21.1	Xtriage
Anisotropy	0.039	Xtriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.34 , 50.8	EDS
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.44$ , $\langle L^2 \rangle = 0.27$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
$F_o, F_c$ correlation	0.96	EDS
Total number of atoms	3941	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	27.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 4.37% of the height of the origin peak. No significant pseudotranslation is detected.*

<sup>1</sup>Intensities estimated from amplitudes.

<sup>2</sup>Theoretical values of  $\langle |L| \rangle$ ,  $\langle L^2 \rangle$  for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

## 5 Model quality [i](#)

### 5.1 Standard geometry [i](#)

Bond lengths and bond angles in the following residue types are not validated in this section: GOL, ZN, NA, LMR, OQL, EDO, DMS

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	0.39	0/3638	0.54	0/4920

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 [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	3539	0	3502	28	0
2	A	1	0	0	0	0
3	A	16	0	24	0	0
4	A	9	0	4	0	0
5	A	6	0	8	3	0
6	A	36	0	54	5	0
7	A	2	0	0	0	0
8	A	332	0	0	3	0
All	All	3941	0	3592	29	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 4.

The worst 5 of 29 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:A:69:PRO:HB3	1:A:422:LEU:HB3	1.78	0.66
1:A:172:VAL:HG21	1:A:182:SER:OG	1.96	0.64
1:A:172:VAL:HG21	1:A:182:SER:CB	2.29	0.63
1:A:123:ILE:HG23	1:A:126:GLU:HB3	1.80	0.62
1:A:45:ILE:HB	1:A:154[B]:ILE:HG22	1.83	0.61

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	436/445 (98%)	416 (95%)	15 (3%)	5 (1%)	14 3

All (5) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	306	THR
1	A	424	GLU
1	A	163	PHE
1	A	168	PHE
1	A	170	HIS

### 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	393/396 (99%)	387 (98%)	6 (2%)	65 51

5 of 6 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	172	VAL
1	A	315	ASN
1	A	272	LYS
1	A	170	HIS
1	A	309	SER

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (2) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	26	ASN
1	A	29	GLN

### 5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

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

1 non-standard protein/DNA/RNA residue is modelled in this entry.

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. 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  $|Z| > 2$  is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\# Z  > 2$	Counts	RMSZ	$\# Z  > 2$
1	0QL	A	165	1	8,9,10	0.88	0	5,9,11	1.40	1 (20%)

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
1	0QL	A	165	1	-	5/5/8/10	-

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	165	0QL	CB-SG-SD	2.56	110.46	103.82

There are no chirality outliers.

All (5) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	A	165	0QL	N-CA-CB-SG
1	A	165	0QL	SD-C1-C2-N3
1	A	165	0QL	C1-SD-SG-CB
1	A	165	0QL	CA-CB-SG-SD
1	A	165	0QL	C2-C1-SD-SG

There are no ring outliers.

1 monomer is involved in 1 short contact:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
1	A	165	0QL	1	0

## 5.5 Carbohydrates [i](#)

There are no monosaccharides in this entry.

## 5.6 Ligand geometry [i](#)

Of 18 ligands modelled in this entry, 3 are monoatomic - leaving 15 for Mogul analysis.

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. 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  $|Z| > 2$  is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	# Z  > 2	Counts	RMSZ	# Z  > 2
6	EDO	A	514	-	3,3,3	0.51	0	2,2,2	0.32	0
6	EDO	A	508	-	3,3,3	0.46	0	2,2,2	0.46	0
6	EDO	A	515	-	3,3,3	0.50	0	2,2,2	0.35	0
6	EDO	A	510	-	3,3,3	0.42	0	2,2,2	0.63	0
6	EDO	A	511	-	3,3,3	0.46	0	2,2,2	0.34	0
5	GOL	A	507	-	5,5,5	0.33	0	5,5,5	0.24	0
3	DMS	A	503	-	3,3,3	0.66	0	3,3,3	0.28	0
6	EDO	A	516	-	3,3,3	0.48	0	2,2,2	0.42	0
6	EDO	A	512	-	3,3,3	0.43	0	2,2,2	0.54	0
4	LMR	A	506	-	2,8,8	0.78	0	3,10,10	1.47	0
6	EDO	A	509	-	3,3,3	0.43	0	2,2,2	0.45	0
3	DMS	A	505	-	3,3,3	0.69	0	3,3,3	0.42	0
3	DMS	A	502	-	3,3,3	0.69	0	3,3,3	0.53	0
6	EDO	A	513	-	3,3,3	0.49	0	2,2,2	0.38	0
3	DMS	A	504	-	3,3,3	0.65	0	3,3,3	0.24	0

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
6	EDO	A	514	-	-	1/1/1/1	-
6	EDO	A	508	-	-	1/1/1/1	-
6	EDO	A	515	-	-	0/1/1/1	-
6	EDO	A	510	-	-	1/1/1/1	-
6	EDO	A	511	-	-	0/1/1/1	-
5	GOL	A	507	-	-	1/4/4/4	-
6	EDO	A	516	-	-	0/1/1/1	-
6	EDO	A	512	-	-	0/1/1/1	-
4	LMR	A	506	-	-	0/2/8/8	-
6	EDO	A	509	-	-	0/1/1/1	-
6	EDO	A	513	-	-	0/1/1/1	-

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (4) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
6	A	510	EDO	O1-C1-C2-O2

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Mol	Chain	Res	Type	Atoms
5	A	507	GOL	C1-C2-C3-O3
6	A	508	EDO	O1-C1-C2-O2
6	A	514	EDO	O1-C1-C2-O2

There are no ring outliers.

4 monomers are involved in 8 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
6	A	514	EDO	1	0
5	A	507	GOL	3	0
6	A	516	EDO	2	0
6	A	513	EDO	2	0

## 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 [i](#)

### 6.1 Protein, DNA and RNA chains [i](#)

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<sup>th</sup> 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 <sup>2</sup> )	Q<0.9
1	A	425/445 (95%)	0.19	36 (8%) 10 12	10, 22, 56, 82	0

The worst 5 of 36 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	163	PHE	13.3
1	A	425	PHE	9.2
1	A	168	PHE	8.6
1	A	170	HIS	6.5
1	A	169	GLY	6.4

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

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<sup>th</sup> 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 <sup>2</sup> )	Q<0.9
1	0QL	A	165	10/11	0.56	0.28	25,51,69,74	0

### 6.3 Carbohydrates [i](#)

There are no monosaccharides in this entry.

### 6.4 Ligands [i](#)

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<sup>th</sup> 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 <sup>2</sup> )	Q<0.9
6	EDO	A	508	4/4	0.70	0.14	33,37,43,56	0
4	LMR	A	506	9/9	0.75	0.24	26,38,43,45	0
6	EDO	A	512	4/4	0.78	0.15	31,39,40,46	0
6	EDO	A	515	4/4	0.78	0.33	38,44,48,49	0
6	EDO	A	513	4/4	0.78	0.26	38,43,45,53	0
5	GOL	A	507	6/6	0.79	0.28	30,37,44,47	0
6	EDO	A	510	4/4	0.85	0.17	27,31,32,38	0
6	EDO	A	511	4/4	0.85	0.18	34,36,39,42	0
6	EDO	A	514	4/4	0.86	0.14	38,43,45,55	0
6	EDO	A	509	4/4	0.91	0.10	24,38,39,42	0
6	EDO	A	516	4/4	0.94	0.10	27,29,45,49	0
7	NA	A	518	1/1	0.95	0.18	40,40,40,40	0
3	DMS	A	502	4/4	0.96	0.11	26,30,38,62	0
3	DMS	A	504	4/4	0.96	0.12	30,33,44,45	0
7	NA	A	517	1/1	0.97	0.22	37,37,37,37	0
3	DMS	A	505	4/4	0.98	0.07	45,47,51,53	0
3	DMS	A	503	4/4	0.98	0.11	31,34,35,40	0
2	ZN	A	501	1/1	1.00	0.07	15,15,15,15	0

## 6.5 Other polymers

There are no such residues in this entry.