

wwPDB X-ray Structure Validation Summary Report (i)

Nov 4, 2023 – 06:08 AM EDT

PDB ID : 4W6U

Title: Crystal Structure of Full-Length Split GFP Mutant E115H/T118H With

Nickel Mediated Crystal Contacts, P 21 21 21 Space Group

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Deposited on : 2014-08-20

Resolution : 2.28 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:

 $Mol Probity \quad : \quad 4.02b\text{--}467$

Mogul: 1.8.5 (274361), CSD as541be (2020)

Xtriage (Phenix) : 1.13 EDS : 2.36

Percentile statistics : 20191225.v01 (using entries in the PDB archive December 25th 2019)

 $Refmac \quad : \quad 5.8.0158$

CCP4 : 7.0.044 (Gargrove)

Ideal geometry (proteins) : Engh & Huber (2001) Ideal geometry (DNA, RNA) : Parkinson et al. (1996)

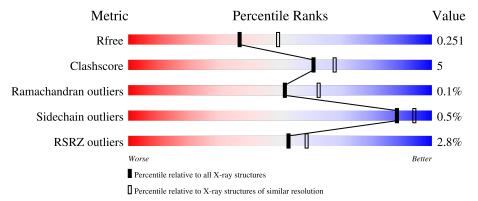
Validation Pipeline (wwPDB-VP) : 2.36

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 2.28 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 $(\# \mathrm{Entries})$	$\begin{array}{c} {\rm Similar\ resolution} \\ (\#{\rm Entries,\ resolution\ range(\mathring{\rm A})}) \end{array}$
R_{free}	130704	6980 (2.30-2.26)
Clashscore	141614	7711 (2.30-2.26)
Ramachandran outliers	138981	7597 (2.30-2.26)
Sidechain outliers	138945	7598 (2.30-2.26)
RSRZ outliers	127900	6849 (2.30-2.26)

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	229	88%	9% •
1	В	229	88%	8% • •
1	С	229	90%	7% •
1	D	229	90%	7% •

The following table lists non-polymeric compounds, carbohydrate monomers and non-standard



residues in protein, DNA, RNA chains that are outliers for geometric or electron-density-fit criteria:

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
3	EDO	A	303	-	-	X	-
4	CIT	С	301	-	X	-	-



2 Entry composition (i)

There are 5 unique types of molecules in this entry. The entry contains 7317 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 fluorescent protein E115H/T118H.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	Λ	223	Total	С	N	О	S	0	0	0
1	A	223	1801	1148	308	342	3	0	U	
1	В	222	Total	С	N	О	S	0	0	0
1	Б	222	1791	1141	306	341	3	0	U	U
1	C	222	Total	С	N	О	S	0	0	0
1			1782	1137	303	339	3	0	U	
1	1 D	223	Total	С	N	О	S	0	0	0
1	ע	223	1797	1144	308	342	3	0	0	

• Molecule 2 is NICKEL (II) ION (three-letter code: NI) (formula: Ni).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
2	A	1	Total Ni 1 1	0	0
2	В	1	Total Ni 1 1	0	0

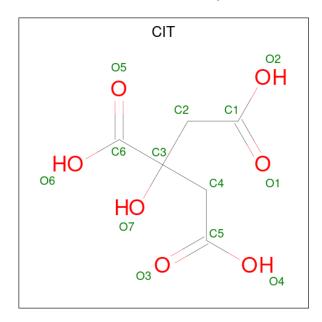
• Molecule 3 is 1,2-ETHANEDIOL (three-letter code: EDO) (formula: $C_2H_6O_2$).





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

• Molecule 4 is CITRIC ACID (three-letter code: CIT) (formula: C₆H₈O₇).



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	С	1	Total C O 13 6 7	0	0

• Molecule 5 is water.



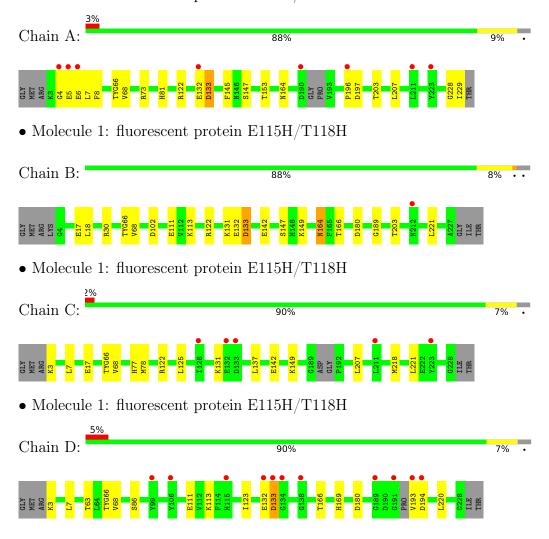
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	A	30	Total O 30 30	0	0
5	В	45	Total O 45 45	0	0
5	С	26	Total O 26 26	0	0
5	D	22	Total O 22 22	0	0



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: fluorescent protein E115H/T118H





4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants	47.64Å 116.58Å 165.98Å	Depositor
a, b, c, α , β , γ	90.00° 90.00° 90.00°	Depositor
Resolution (Å)	82.99 - 2.28	Depositor
resolution (A)	82.99 - 2.28	EDS
% Data completeness	99.3 (82.99-2.28)	Depositor
(in resolution range)	99.3 (82.99-2.28)	EDS
R_{merge}	(Not available)	Depositor
R_{sym}	0.11	Depositor
$< I/\sigma(I) > 1$	1.37 (at 2.27Å)	Xtriage
Refinement program	PHENIX (PHENIX.REFINE: DEV_1555)	Depositor
R, R_{free}	0.210 , 0.250	Depositor
it, it free	0.214 , 0.251	DCC
R_{free} test set	4293 reflections (10.00%)	wwPDB-VP
Wilson B-factor (\mathring{A}^2)	44.6	Xtriage
Anisotropy	0.321	Xtriage
Bulk solvent $k_{sol}(e/Å^3)$, $B_{sol}(Å^2)$	0.38, 50.2	EDS
L-test for twinning ²	$< L >=0.49, < L^2>=0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.94	EDS
Total number of atoms	7317	wwPDB-VP
Average B, all atoms $(Å^2)$	47.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 5.00% of the height of the origin peak. No significant pseudotranslation is detected.

²Theoretical 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.



¹Intensities estimated from amplitudes.

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: CRO, EDO, NI, CIT

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).

Mal	Clasia	Bo	nd lengths	Bond angles		
IVIOI	Mol Chain		# Z > 5	RMSZ	# Z > 5	
1	A	0.52	1/1818 (0.1%)	0.69	$2/2455 \ (0.1\%)$	
1	В	0.50	1/1810 (0.1%)	0.66	0/2448	
1	С	0.45	1/1800 (0.1%)	0.61	0/2433	
1	D	0.47	1/1814 (0.1%)	0.63	0/2449	
All	All	0.48	4/7242 (0.1%)	0.65	$2/9785 \ (0.0\%)$	

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 maintenain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	#Chirality outliers	#Planarity outliers
1	В	0	1

All (4) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(A)	$\operatorname{Ideal}(ext{\AA})$
1	D	68	VAL	N-CA	-7.35	1.31	1.46
1	A	68	VAL	N-CA	-7.21	1.31	1.46
1	В	68	VAL	N-CA	-6.63	1.33	1.46
1	С	68	VAL	N-CA	-6.00	1.34	1.46

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$\mathbf{Observed}(^{o})$	$\operatorname{Ideal}(^{o})$
1	A	68	VAL	N-CA-C	-6.41	93.69	111.00
1	A	197	ASP	CB-CG-OD1	5.07	122.86	118.30

There are no chirality outliers.

All (1) planarity outliers are listed below:



Mol	Chain	Res	Type	Group
1	В	189	GLY	Mainchain

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	1801	0	1750	21	0
1	В	1791	0	1734	20	0
1	С	1782	0	1721	10	0
1	D	1797	0	1742	17	0
2	A	1	0	0	0	0
2	В	1	0	0	0	0
3	A	8	0	12	5	0
4	С	13	0	4	0	0
5	A	30	0	0	1	0
5	В	45	0	0	0	1
5	С	26	0	0	0	0
5	D	22	0	0	1	0
All	All	7317	0	6963	64	1

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 5.

The worst 5 of 64 close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	$\begin{array}{c} {\rm Interatomic} \\ {\rm distance} \ (\rm \mathring{A}) \end{array}$	$egin{aligned} ext{Clash} \ ext{overlap } (ext{Å}) \end{aligned}$
3:A:303:EDO:H12	1:B:102:ASP:HA	1.58	0.85
1:C:131:LYS:O	1:C:137:LEU:HD12	1.85	0.77
1:B:17:GLU:OE1	1:B:122:ARG:NH1	2.20	0.74
1:D:193:VAL:HG13	1:D:194:ASP:N	2.07	0.69
1:A:133:ASP:OD1	1:A:133:ASP:C	2.34	0.66

All (1) symmetry-related close contacts are listed below. The label for Atom-2 includes the symmetry operator and encoded unit-cell translations to be applied.



Atom-1	Atom-2	$\begin{array}{c} {\rm Interatomic} \\ {\rm distance} \ ({\rm \AA}) \end{array}$	Clash overlap (Å)
5:B:404:HOH:O	5:B:413:HOH:O[1_455]	2.12	0.08

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	Perce	entiles
1	A	216/229 (94%)	209 (97%)	6 (3%)	1 (0%)	29	34
1	В	$217/229 \ (95\%)$	212 (98%)	5 (2%)	0	100	100
1	С	$215/229 \ (94\%)$	211 (98%)	4 (2%)	0	100	100
1	D	$216/229 \ (94\%)$	213 (99%)	3 (1%)	0	100	100
All	All	864/916 (94%)	845 (98%)	18 (2%)	1 (0%)	51	63

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	6	GLU

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	193/197 (98%)	192 (100%)	1 (0%)	88	94	
1	В	192/197 (98%)	190 (99%)	2 (1%)	76	86	
1	С	190/197 (96%)	190 (100%)	0	100	100	
1	D	192/197 (98%)	191 (100%)	1 (0%)	88	94	

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Mol	Chain	Analysed	Analysed Rotameric		Percentiles
All	All	767/788 (97%)	763 (100%)	4 (0%)	88 94

All (4) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	133	ASP
1	В	133	ASP
1	В	164	ASN
1	D	133	ASP

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	164	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)

4 non-standard protein/DNA/RNA residues are 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 Re	Dog	Res Link	Bond lengths			Bond angles		
$oxed{egin{array}{c c} Mol & Type \\ \hline \end{array}}$	nes		Counts		RMSZ	# Z > 2	Counts	RMSZ	# Z >2	
1	CRO	В	66	1	23,23,24	3.75	7 (30%)	30,32,34	4.09	8 (26%)
1	CRO	D	66	1	23,23,24	4.10	7 (30%)	30,32,34	4.12	7 (23%)
1	CRO	A	66	1	23,23,24	3.77	7 (30%)	30,32,34	3.21	9 (30%)
1	CRO	С	66	1	23,23,24	3.87	8 (34%)	30,32,34	3.31	7 (23%)

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.

	\mathbf{Mol}	Type	Chain	Res	Link	Chirals	Torsions	Rings
	1	CRO	В	66	1	-	0/12/31/32	0/2/2/2
	1	CRO	D	66	1	-	0/12/31/32	0/2/2/2
	1	CRO	A	66	1	-	0/12/31/32	0/2/2/2
ĺ	1	CRO	С	66	1	-	0/12/31/32	0/2/2/2

The worst 5 of 29 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(A)	Ideal(A)
1	D	66	CRO	CB2-CA2	17.31	1.49	1.35
1	С	66	CRO	CB2-CA2	15.20	1.47	1.35
1	В	66	CRO	CB2-CA2	15.04	1.47	1.35
1	A	66	CRO	CB2-CA2	13.49	1.46	1.35
1	A	66	CRO	CA2-C2	-7.31	1.41	1.48

The worst 5 of 31 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$Observed(^o)$	$Ideal(^{o})$
1	D	66	CRO	CA2-C2-N3	15.04	110.48	103.37
1	В	66	CRO	O2-C2-CA2	-13.62	123.31	130.96
1	В	66	CRO	CA2-C2-N3	13.55	109.78	103.37
1	D	66	CRO	O2-C2-CA2	-11.86	124.30	130.96
1	С	66	CRO	CA2-C2-N3	11.35	108.74	103.37

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

No monomer is involved in short contacts.

5.5 Carbohydrates (i)

There are no monosaccharides in this entry.

5.6 Ligand geometry (i)

Of 5 ligands modelled in this entry, 2 are monoatomic - leaving 3 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	Tuno	Chain	Res	Link	Во	Bond lengths			ond ang	les
MIOI	Type	Chain	nes	Lilik	Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z > 2
3	EDO	A	303	-	3,3,3	0.32	0	2,2,2	0.37	0
4	CIT	С	301	-	12,12,12	3.63	5 (41%)	17,17,17	2.79	7 (41%)
3	EDO	A	302	-	3,3,3	0.31	0	2,2,2	0.33	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
3	EDO	A	303	-	-	1/1/1/1	-
4	CIT	С	301	-	-	12/16/16/16	-
3	EDO	A	302	-	-	1/1/1/1	-

All (5) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(A)	$\operatorname{Ideal}(\text{\AA})$
4	С	301	CIT	C3-C6	-10.24	1.42	1.53
4	С	301	CIT	O7-C3	-3.82	1.35	1.43
4	С	301	CIT	C2-C3	-3.78	1.49	1.53
4	С	301	CIT	C4-C3	-3.61	1.49	1.53
4	С	301	CIT	O6-C6	-2.08	1.22	1.30

The worst 5 of 7 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$Observed(^{o})$	$\operatorname{Ideal}(^{o})$
4	С	301	CIT	O7-C3-C6	-9.21	95.94	108.86
4	С	301	CIT	C4-C3-C6	3.03	116.62	110.11
4	С	301	CIT	O1-C1-C2	-2.82	114.69	122.94
4	С	301	CIT	O2-C1-C2	2.40	122.05	114.35
4	С	301	CIT	O3-C5-C4	-2.37	116.02	122.94

There are no chirality outliers.

5 of 14 torsion outliers are listed below:



Mol	Chain	Res	Type	Atoms
4	С	301	CIT	C1-C2-C3-O7
4	С	301	CIT	C2-C3-C4-C5
4	С	301	CIT	C6-C3-C4-C5
4	С	301	CIT	O7-C3-C6-O6
4	С	301	CIT	C1-C2-C3-C4

There are no ring outliers.

1 monomer is involved in 5 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	A	303	EDO	5	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^{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	222/229 (96%)	0.17	8 (3%) 42	48	27, 40, 64, 125	0
1	В	221/229 (96%)	0.02	1 (0%) 91	93	26, 40, 60, 125	0
1	С	221/229 (96%)	0.16	5 (2%) 60	66	24, 47, 70, 101	0
1	D	222/229 (96%)	0.30	11 (4%) 28	34	32, 53, 82, 116	0
All	All	886/916 (96%)	0.16	25 (2%) 53	59	24, 45, 75, 125	0

The worst 5 of 25 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	4	GLY	12.1
1	С	133	ASP	5.6
1	D	133	ASP	5.3
1	С	223	TYR	4.6
1	D	193	VAL	3.9

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^{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	$\mathbf{B} ext{-}\mathbf{factors}(\mathbf{\mathring{A}}^2)$	Q<0.9
1	CRO	A	66	22/23	0.94	0.13	24,29,36,39	0
1	CRO	В	66	22/23	0.94	0.14	22,26,33,37	0
1	CRO	D	66	22/23	0.94	0.11	30,32,43,43	0
1	CRO	С	66	22/23	0.95	0.11	27,32,39,43	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^{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	$\mathbf{B} ext{-}\mathbf{factors}(\mathbf{\mathring{A}}^2)$	Q < 0.9
2	NI	В	301	1/1	0.68	0.10	63,63,63,63	0
3	EDO	A	302	4/4	0.78	0.19	54,55,55,56	0
3	EDO	A	303	4/4	0.82	0.21	48,52,56,57	0
4	CIT	С	301	13/13	0.84	0.23	39,49,61,63	0
2	NI	A	301	1/1	0.94	0.09	70,70,70,70	0

6.5 Other polymers (i)

There are no such residues in this entry.

