



wwPDB X-ray Structure Validation Summary Report ⓘ

Sep 8, 2025 – 02:06 PM EDT

PDB ID : 9NCX / pdb_00009ncx
Title : NitrOFF1 "ON" State
Authors : Smailys, J.; Zhang, Y.
Deposited on : 2025-02-17
Resolution : 3.00 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 ⓘ 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 ⓘ](#)) were used in the production of this report:

MolProbity : 4-5-2 with Phenix2.0rc1
Mogul : 2022.3.0, CSD as543be (2022)
Xtriage (Phenix) : 2.0rc1
EDS : 3.0
Percentile statistics : 20231227.v01 (using entries in the PDB archive December 27th 2023)
CCP4 : 9.0.006 (Gargrove)
Density-Fitness : 1.0.12
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : 2.45.1

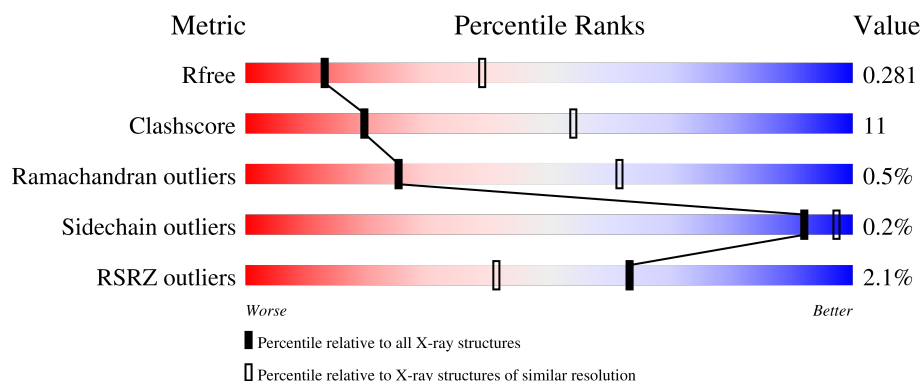
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 3.00 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}	164625	2511 (3.00-3.00)
Clashscore	180529	2866 (3.00-3.00)
Ramachandran outliers	177936	2778 (3.00-3.00)
Sidechain outliers	177891	2781 (3.00-3.00)
RSRZ outliers	164620	2523 (3.00-3.00)

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 $\leq 5\%$. 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	385	<div> <div>2%</div> <div> <div></div> <div>71%</div> <div>23%</div> <div>• 5%</div> </div> </div>
1	B	385	<div> <div>2%</div> <div> <div></div> <div>66%</div> <div>27%</div> <div>• 5%</div> </div> </div>
1	C	385	<div> <div>2%</div> <div> <div></div> <div>71%</div> <div>23%</div> <div>• 5%</div> </div> </div>
1	D	385	<div> <div>2%</div> <div> <div></div> <div>68%</div> <div>25%</div> <div>• 5%</div> </div> </div>

2 Entry composition

There is only 1 type of molecule in this entry. The entry contains 11584 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.

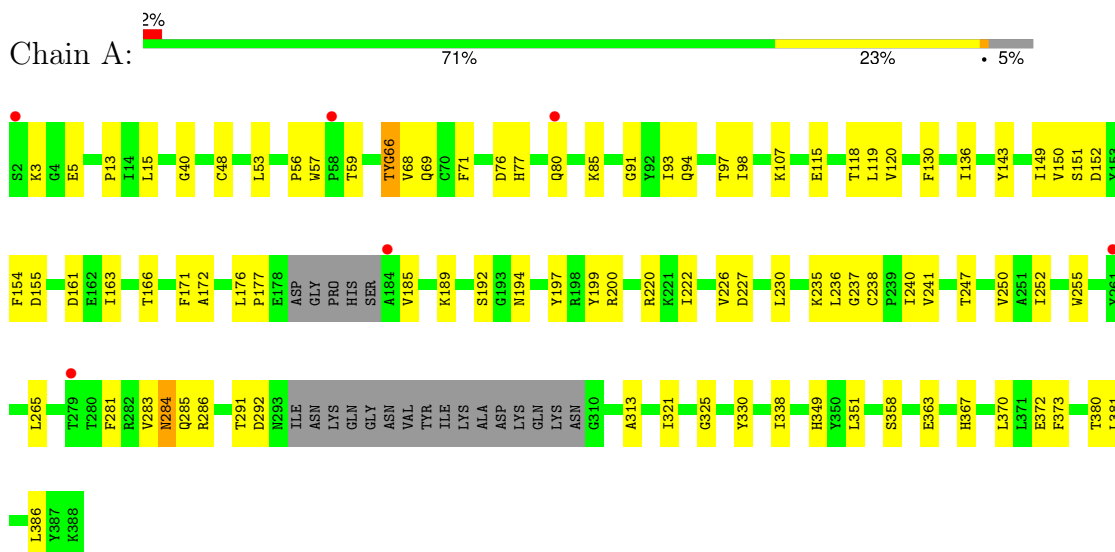
- Molecule 1 is a protein called Engineered fluorescent biosensor NitrOFF1 "ON" State.

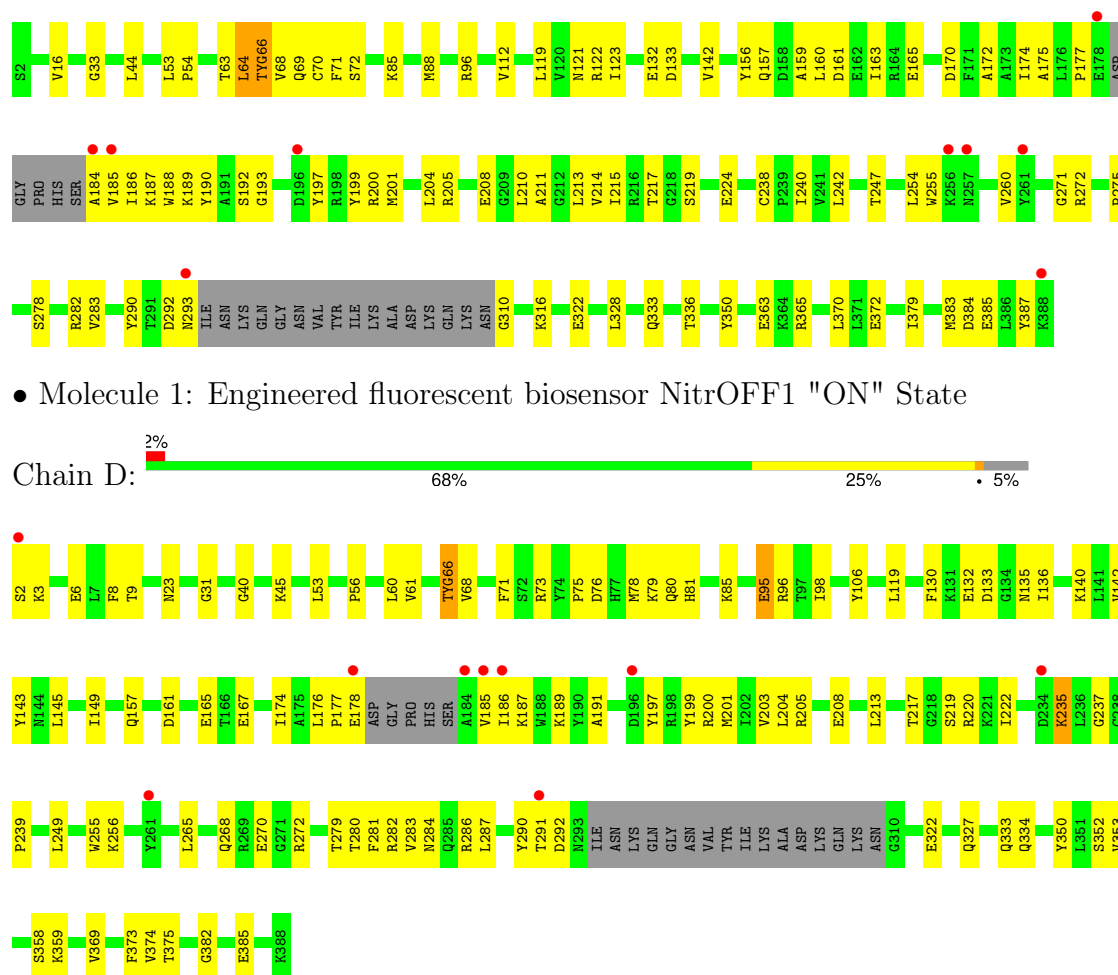
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	364	Total	C	N	O	S	0	0	0
			2896	1848	486	554	8			
1	B	364	Total	C	N	O	S	0	0	0
			2896	1848	486	554	8			
1	C	364	Total	C	N	O	S	0	0	0
			2896	1848	486	554	8			
1	D	364	Total	C	N	O	S	0	0	0
			2896	1848	486	554	8			

3 Residue-property plots

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: Engineered fluorescent biosensor NitrOFF1 "ON" State





• Molecule 1: Engineered fluorescent biosensor NitroFF1 "ON" State

4 Data and refinement statistics

Property	Value	Source
Space group	C 2 2 21	Depositor
Cell constants a, b, c, α , β , γ	132.91Å 188.35Å 188.28Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	56.77 – 3.00 56.77 – 3.00	Depositor EDS
% Data completeness (in resolution range)	93.1 (56.77-3.00) 93.1 (56.77-3.00)	Depositor EDS
R_{merge}	0.22	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.48 (at 3.01Å)	Xtriage
Refinement program	PHENIX (1.18.2_3874: ???)	Depositor
R, R_{free}	0.238 , 0.280 0.239 , 0.281	Depositor DCC
R_{free} test set	2000 reflections (4.21%)	wwPDB-VP
Wilson B-factor (Å ²)	54.5	Xtriage
Anisotropy	0.258	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.35 , 45.5	EDS
L-test for twinning ²	$\langle L \rangle = 0.44$, $\langle L^2 \rangle = 0.27$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.89	EDS
Total number of atoms	11584	wwPDB-VP
Average B, all atoms (Å ²)	49.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The analyses of the Patterson function reveals a significant off-origin peak that is 39.24 % of the origin peak, indicating pseudo-translational symmetry. The chance of finding a peak of this or larger height randomly in a structure without pseudo-translational symmetry is equal to 3.2696e-04. The detected translational NCS is most likely also responsible for the elevated intensity ratio.*

¹Intensities estimated from amplitudes.

²Theoretical 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

5.1 Standard geometry

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

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	0.27	0/2932	0.61	0/3965
1	B	0.22	0/2932	0.64	4/3965 (0.1%)
1	C	0.22	0/2932	0.60	1/3965 (0.0%)
1	D	0.22	0/2932	0.59	2/3965 (0.1%)
All	All	0.23	0/11728	0.61	7/15860 (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	1
1	B	0	3
All	All	0	4

There are no bond length outliers.

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

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	B	132	GLU	CA-C-N	6.06	128.72	120.54
1	B	132	GLU	C-N-CA	6.06	128.72	120.54
1	D	167	GLU	CB-CG-CD	5.42	121.82	112.60
1	B	291	THR	CA-C-N	-5.23	111.73	122.82
1	B	291	THR	C-N-CA	-5.23	111.73	122.82

There are no chirality outliers.

All (4) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	255	TRP	Peptide
1	B	281	PHE	Peptide
1	B	292	ASP	Peptide
1	B	3	LYS	Peptide

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	2896	0	2855	57	2
1	B	2896	0	2854	80	1
1	C	2896	0	2855	73	0
1	D	2896	0	2854	65	1
All	All	11584	0	11418	263	2

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 11.

The worst 5 of 263 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:66:CRO:HA1	1:C:68:VAL:HG22	1.50	0.92
1:D:220:ARG:HH12	1:D:281:PHE:H	1.17	0.89
1:A:220:ARG:NH2	1:A:281:PHE:O	2.05	0.88
1:A:149:ILE:HB	1:A:386:LEU:HD21	1.59	0.84
1:C:177:PRO:HA	1:C:185:VAL:O	1.83	0.77

All (2) 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	Interatomic distance (Å)	Clash overlap (Å)
1:A:115:GLU:OE1	1:B:122:ARG:NH2[4_555]	2.11	0.09
1:A:200:ARG:NH2	1:D:385:GLU:OE1[5_555]	2.19	0.01

5.3 Torsion angles ⓘ

5.3.1 Protein backbone ⓘ

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	355/385 (92%)	338 (95%)	13 (4%)	4 (1%)	12	44
1	B	355/385 (92%)	340 (96%)	13 (4%)	2 (1%)	22	57
1	C	355/385 (92%)	345 (97%)	10 (3%)	0	100	100
1	D	355/385 (92%)	345 (97%)	9 (2%)	1 (0%)	37	70
All	All	1420/1540 (92%)	1368 (96%)	45 (3%)	7 (0%)	25	61

5 of 7 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	151	SER
1	A	155	ASP
1	A	236	LEU
1	B	385	GLU
1	A	284	ASN

5.3.2 Protein sidechains ⓘ

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	312/330 (94%)	312 (100%)	0	100	100
1	B	312/330 (94%)	311 (100%)	1 (0%)	91	96
1	C	312/330 (94%)	311 (100%)	1 (0%)	91	96
1	D	312/330 (94%)	312 (100%)	0	100	100
All	All	1248/1320 (94%)	1246 (100%)	2 (0%)	92	97

All (2) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	69	GLN
1	C	64	LEU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 9 such sidechains are listed below:

Mol	Chain	Res	Type
1	C	257	ASN
1	D	105	ASN
1	B	69	GLN
1	B	94	GLN
1	B	135	ASN

5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

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

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	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\# Z > 2$	Counts	RMSZ	$\# Z > 2$
1	CRO	D	66	1	22,23,24	0.56	0	30,32,34	1.22	6 (20%)
1	CRO	B	66	1	22,23,24	0.57	0	30,32,34	1.17	5 (16%)
1	CRO	C	66	1	22,23,24	0.57	0	30,32,34	1.30	5 (16%)
1	CRO	A	66	1	22,23,24	1.22	2 (9%)	30,32,34	2.32	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.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
1	CRO	D	66	1	-	4/12/31/32	0/2/2/2
1	CRO	B	66	1	-	4/12/31/32	0/2/2/2
1	CRO	C	66	1	-	4/12/31/32	0/2/2/2
1	CRO	A	66	1	-	4/12/31/32	0/2/2/2

All (2) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	66	CRO	C1-N2	4.10	1.38	1.32
1	A	66	CRO	OG1-CB1	2.45	1.49	1.43

The worst 5 of 23 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	66	CRO	OG1-CB1-CA1	6.46	122.76	109.00
1	A	66	CRO	CA1-C1-N3	-6.09	117.48	124.69
1	A	66	CRO	CA1-C1-N2	5.48	131.32	123.88
1	C	66	CRO	CA1-C1-N2	-3.78	118.75	123.88
1	D	66	CRO	C2-N3-C1	-3.48	106.46	108.07

There are no chirality outliers.

5 of 16 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	A	66	CRO	N1-CA1-CB1-OG1
1	A	66	CRO	C3-CA3-N3-C2
1	B	66	CRO	N2-C1-CA1-CB1
1	B	66	CRO	N3-C1-CA1-CB1
1	B	66	CRO	N2-CA2-CB2-CG2

There are no ring outliers.

4 monomers are involved in 11 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
1	D	66	CRO	1	0
1	B	66	CRO	1	0
1	C	66	CRO	3	0
1	A	66	CRO	6	0

5.5 Carbohydrates [i](#)

There are no oligosaccharides in this entry.

5.6 Ligand geometry [i](#)

There are no ligands in this entry.

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, 95th 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	363/385 (94%)	0.07	6 (1%) 69 47	28, 47, 77, 95	0
1	B	363/385 (94%)	0.02	6 (1%) 69 47	26, 44, 74, 105	0
1	C	363/385 (94%)	0.01	9 (2%) 58 36	24, 45, 74, 101	0
1	D	363/385 (94%)	0.11	9 (2%) 58 36	27, 47, 75, 109	0
All	All	1452/1540 (94%)	0.05	30 (2%) 63 41	24, 46, 76, 109	0

The worst 5 of 30 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	D	185	VAL	7.3
1	D	184	ALA	6.0
1	B	293	ASN	5.9
1	D	186	ILE	5.1
1	C	178	GLU	3.6

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, 95th 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
1	CRO	C	66	22/23	0.90	0.16	40,51,58,65	0
1	CRO	A	66	22/23	0.91	0.19	30,46,64,71	0
1	CRO	B	66	22/23	0.92	0.17	33,59,72,80	0
1	CRO	D	66	22/23	0.92	0.15	36,48,62,72	0

6.3 Carbohydrates [i](#)

There are no oligosaccharides 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.