



Full wwPDB X-ray Structure Validation Report ⓘ

Feb 1, 2016 – 04:30 PM GMT

PDB ID : 4F9V
Title : Structure of C113A/C136A mutant variant of glycosylated glutaminyl cyclase from *Drosophila melanogaster*
Authors : Kolenko, P.; Koch, B.; Ruiz-Carilo, D.; Stubbs, M.T.
Deposited on : 2012-05-21
Resolution : 2.10 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
<http://wwpdb.org/validation/2016/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
Mogul : 1.7 (RC4), CSD as536be (2015)
Xtriage (Phenix) : 1.9-1692
EDS : rb-20026688
Percentile statistics : 20151230.v01 (using entries in the PDB archive December 30th 2015)
Refmac : 5.8.0135
CCP4 : 6.5.0
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : trunk26865

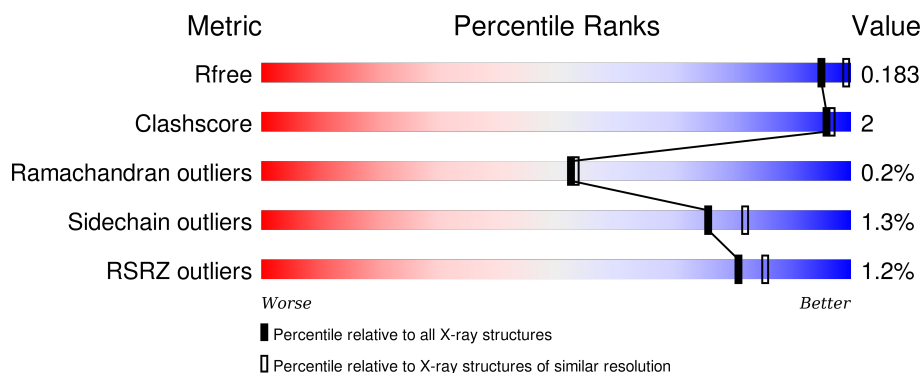
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

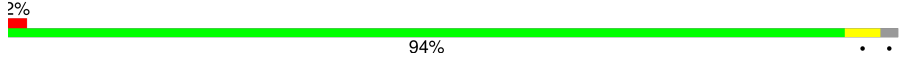

The reported resolution of this entry is 2.10 Å.

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}	91344	3939 (2.10-2.10)
Clashscore	102246	4460 (2.10-2.10)
Ramachandran outliers	100387	4413 (2.10-2.10)
Sidechain outliers	100360	4414 (2.10-2.10)
RSRZ outliers	91569	3948 (2.10-2.10)

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. 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	312	
1	B	312	

2 Entry composition

There are 7 unique types of molecules in this entry. The entry contains 5727 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 CG32412.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	307	Total	C	N	O	S	0	1	0
			2453	1557	440	454	2			
1	B	299	Total	C	N	O	S	0	1	0
			2414	1538	432	442	2			

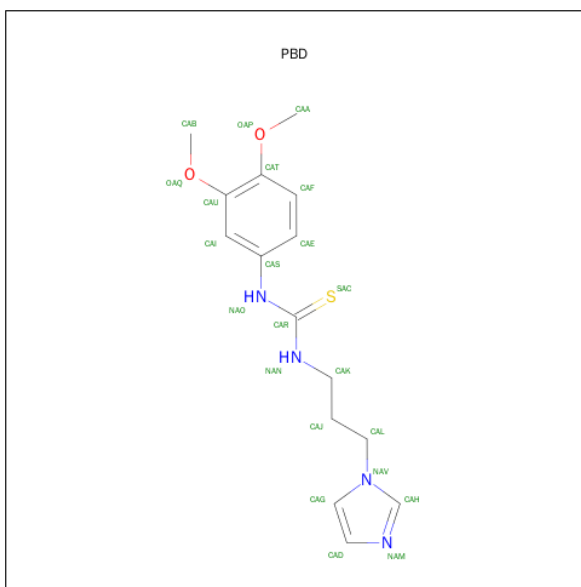
There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	113	ALA	CYS	ENGINEERED MUTATION	UNP Q9VRQ9
A	136	ALA	CYS	ENGINEERED MUTATION	UNP Q9VRQ9
B	113	ALA	CYS	ENGINEERED MUTATION	UNP Q9VRQ9
B	136	ALA	CYS	ENGINEERED MUTATION	UNP Q9VRQ9

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

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

- Molecule 3 is 1-(3,4-DIMETHOXYPHENYL)-3-[3-(1H-IMIDAZOL-1-YL)PROPYL]THIO UREA (three-letter code: PBD) (formula: C₁₅H₂₀N₄O₂S).



Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
3	A	1	Total	C	N	O	S	0	0
			22	15	4	2	1		
3	B	1	Total	C	N	O	S	0	0
			22	15	4	2	1		

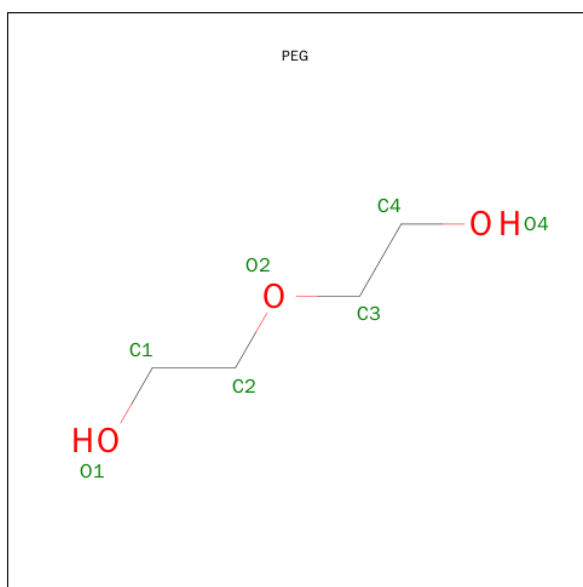
- Molecule 4 is a polymer of unknown type called SUGAR (7-MER).

Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
4	A	7	Total	C	N	O	0	0
			83	46	2	35		

- Molecule 5 is a polymer of unknown type called SUGAR (3-MER).

Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
5	B	3	Total	C	N	O	0	0
			39	22	2	15		

- Molecule 6 is DI(HYDROXYETHYL)ETHER (three-letter code: PEG) (formula: C₄H₁₀O₃).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
6	B	1	Total	C	O	0	0
			7	4	3		

- Molecule 7 is water.

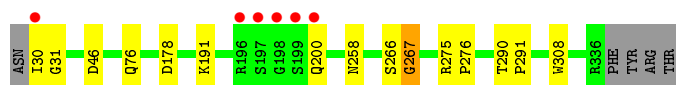
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
7	A	386	Total	O	0	0
			386	386		
7	B	299	Total	O	0	0
			299	299		

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 errors 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 ($\text{RSRZ} > 2$). 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: CG32412

Chain A:  94%



● Molecule 1: CG32412

Chain B:  92%



4 Data and refinement statistics

Property	Value	Source
Space group	P 65	Depositor
Cell constants a, b, c, α , β , γ	170.87Å 170.87Å 57.24Å 90.00° 90.00° 120.00°	Depositor
Resolution (Å)	50.00 – 2.10 47.55 – 2.10	Depositor EDS
% Data completeness (in resolution range)	98.5 (50.00-2.10) 98.5 (47.55-2.10)	Depositor EDS
R_{merge}	0.11	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	2.03 (at 2.10Å)	Xtriage
Refinement program	REFMAC 5.5.0110	Depositor
R, R_{free}	0.174 , 0.215 0.178 , 0.183	Depositor DCC
R_{free} test set	2810 reflections (5.37%)	DCC
Wilson B-factor (Å ²)	25.9	Xtriage
Anisotropy	0.270	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.30 , 41.7	EDS
Estimated twinning fraction	0.032 for h,-h-k,-l	Xtriage
L-test for twinning ²	$\langle L \rangle = 0.49$, $\langle L^2 \rangle = 0.33$	Xtriage
Outliers	0 of 55221 reflections	Xtriage
F_o, F_c correlation	0.95	EDS
Total number of atoms	5727	wwPDB-VP
Average B, all atoms (Å ²)	27.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 2.61% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical values of $\langle |L| \rangle$, $\langle L^2 \rangle$ for acentric reflections are 0.5, 0.375 respectively for untwinned datasets, and 0.333, 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: ZN, BMA, NAG, PBD, PEG, MAN

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	0.74	0/2516	0.72	0/3418
1	B	0.65	0/2475	0.70	1/3363 (0.0%)
All	All	0.70	0/4991	0.71	1/6781 (0.0%)

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	B	235	ASP	CB-CG-OD1	5.31	123.08	118.30

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	2453	0	2392	7	0
1	B	2414	0	2347	5	0
2	A	1	0	0	0	0
2	B	1	0	0	0	0
3	A	22	0	20	0	0
3	B	22	0	20	1	0
4	A	83	0	70	1	0
5	B	39	0	34	0	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
6	B	7	0	10	2	0
7	A	386	0	0	4	0
7	B	299	0	0	1	0
All	All	5727	0	4893	15	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 2.

All (15) 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:262:LEU:HD22	6:B:406:PEG:H32	1.79	0.64
1:B:224[B]:ASN:OD1	1:B:258:ASN:OD1	2.23	0.56
1:B:196:ARG:HD3	1:B:207:ASN:HD21	1.70	0.56
7:A:595:HOH:O	6:B:406:PEG:H22	2.07	0.55
1:A:191:LYS:HE3	7:A:552:HOH:O	2.09	0.53
3:B:402:PBD:HNAN	3:B:402:PBD:CAE	2.23	0.50
1:A:30:ILE:HG13	1:A:31:GLY:N	2.28	0.48
1:A:76:GLN:NE2	7:A:853:HOH:O	2.37	0.47
1:A:275:ARG:HB3	1:A:276:PRO:HD3	2.02	0.41
1:B:290:THR:HA	1:B:291:PRO:C	2.41	0.41
1:A:46[B]:ASP:OD2	7:A:736:HOH:O	2.22	0.41
4:A:406:MAN:H2	4:A:407:MAN:H2	1.90	0.41
1:A:290:THR:HA	1:A:291:PRO:C	2.42	0.40
1:A:266:SER:C	1:A:267:GLY:O	2.60	0.40
1:B:35:ARG:HG2	7:B:703:HOH:O	2.21	0.40

There are no symmetry-related clashes.

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	306/312 (98%)	292 (95%)	13 (4%)	1 (0%)	46	45
1	B	296/312 (95%)	283 (96%)	13 (4%)	0	100	100
All	All	602/624 (96%)	575 (96%)	26 (4%)	1 (0%)	52	53

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	267	GLY

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	265/269 (98%)	261 (98%)	4 (2%)	72	78
1	B	260/269 (97%)	257 (99%)	3 (1%)	78	84
All	All	525/538 (98%)	518 (99%)	7 (1%)	76	82

All (7) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	178	ASP
1	A	200	GLN
1	A	258	ASN
1	A	308	TRP
1	B	195	LYS
1	B	249	ARG
1	B	308	TRP

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (3) such sidechains are listed below:

Mol	Chain	Res	Type
1	B	107	ASN
1	B	151	GLN
1	B	207	ASN

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 ⓘ

10 carbohydrates 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	$\# Z > 2$
4	NAG	A	403	1,4	14,14,15	0.76	0	15,19,21	0.88	0
4	NAG	A	404	4	14,14,15	0.66	0	15,19,21	0.95	1 (6%)
4	BMA	A	405	4	11,11,12	0.70	0	14,15,17	0.89	0
4	MAN	A	406	4	11,11,12	0.60	0	14,15,17	1.38	2 (14%)
4	MAN	A	407	4	11,11,12	0.71	0	14,15,17	1.64	3 (21%)
4	MAN	A	408	4	11,11,12	0.53	0	14,15,17	0.86	1 (7%)
4	MAN	A	409	4	11,11,12	0.46	0	14,15,17	1.01	1 (7%)
5	NAG	B	403	1,5	14,14,15	0.68	0	15,19,21	0.82	0
5	NAG	B	404	5	14,14,15	0.39	0	15,19,21	0.86	1 (6%)
5	BMA	B	405	5	11,11,12	0.58	0	14,15,17	0.78	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
4	NAG	A	403	1,4	-	0/6/23/26	0/1/1/1
4	NAG	A	404	4	-	0/6/23/26	0/1/1/1
4	BMA	A	405	4	-	0/2/19/22	0/1/1/1
4	MAN	A	406	4	-	0/2/19/22	0/1/1/1

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Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
4	MAN	A	407	4	-	0/2/19/22	0/1/1/1
4	MAN	A	408	4	-	0/2/19/22	0/1/1/1
4	MAN	A	409	4	-	0/2/19/22	0/1/1/1
5	NAG	B	403	1,5	-	0/6/23/26	0/1/1/1
5	NAG	B	404	5	-	0/6/23/26	0/1/1/1
5	BMA	B	405	5	-	0/2/19/22	0/1/1/1

There are no bond length outliers.

All (9) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
4	A	407	MAN	O5-C1-C2	-2.96	106.06	110.86
4	A	404	NAG	O4-C4-C3	-2.07	105.69	110.34
4	A	406	MAN	C2-C3-C4	2.01	114.46	111.04
4	A	409	MAN	C1-O5-C5	2.09	114.90	112.25
5	B	404	NAG	O5-C5-C6	2.15	112.00	107.35
4	A	408	MAN	C1-O5-C5	2.18	115.01	112.25
4	A	406	MAN	O2-C2-C1	2.31	113.83	109.21
4	A	407	MAN	C1-C2-C3	2.86	112.92	109.54
4	A	407	MAN	C3-C4-C5	3.41	116.14	110.20

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

2 monomers are involved in 1 short contact:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	A	406	MAN	1	0
4	A	407	MAN	1	0

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	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z > 2
3	PBD	A	402	2	22,23,23	0.96	0	27,29,29	1.16	3 (11%)
3	PBD	B	402	2	22,23,23	0.93	2 (9%)	27,29,29	1.62	5 (18%)
6	PEG	B	406	-	6,6,6	0.58	0	5,5,5	0.53	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	PBD	A	402	2	-	0/15/15/15	0/2/2/2
3	PBD	B	402	2	-	0/15/15/15	0/2/2/2
6	PEG	B	406	-	-	0/4/4/4	0/0/0/0

All (2) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	B	402	PBD	CAG-NAV	-2.14	1.33	1.37
3	B	402	PBD	CAR-SAC	2.44	1.73	1.68

All (8) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
3	B	402	PBD	CAJ-CAK-NAN	-3.40	102.25	112.19
3	A	402	PBD	SAC-CAR-NAO	-2.64	116.19	124.22
3	B	402	PBD	CAS-NAO-CAR	-2.34	121.82	128.60
3	B	402	PBD	CAA-OAP-CAT	2.49	121.32	117.54
3	A	402	PBD	NAO-CAR-NAN	2.56	120.61	114.34
3	A	402	PBD	CAK-NAN-CAR	2.83	128.78	124.60
3	B	402	PBD	CAK-NAN-CAR	3.60	129.92	124.60
3	B	402	PBD	CAB-OAQ-CAU	3.90	123.46	117.54

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

2 monomers are involved in 3 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	B	402	PBD	1	0
6	B	406	PEG	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, 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	307/312 (98%)	-0.70	6 (1%) 68 73	13, 21, 37, 60	1 (0%)
1	B	299/312 (95%)	-0.52	1 (0%) 94 95	16, 27, 43, 52	5 (1%)
All	All	606/624 (97%)	-0.61	7 (1%) 81 85	13, 23, 42, 60	6 (0%)

All (7) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	B	204	ALA	3.8
1	A	200	GLN	3.5
1	A	198	GLY	3.0
1	A	196	ARG	2.8
1	A	197	SER	2.7
1	A	199	SER	2.2
1	A	30	ILE	2.1

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](#)

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. LLDF column lists the quality of electron density of the group with respect to its neighbouring residues in protein, DNA or RNA chains. 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	LLDF	B-factors(Å ²)	Q<0.9
4	NAG	A	403	14/15	0.97	0.07	-0.41	18,22,25,28	0

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Mol	Type	Chain	Res	Atoms	RSCC	RSR	LLDF	B-factors(\AA^2)	Q<0.9
4	BMA	A	405	11/12	0.84	0.13	-	58,66,71,72	0
4	MAN	A	408	11/12	0.77	0.16	-	73,75,76,76	0
4	MAN	A	406	11/12	0.86	0.16	-	77,81,82,84	0
4	NAG	A	404	14/15	0.96	0.08	-	27,34,42,49	0
4	MAN	A	409	11/12	0.77	0.24	-	76,78,80,80	0
5	NAG	B	403	14/15	0.94	0.10	-	34,36,41,45	0
5	BMA	B	405	11/12	0.58	0.30	-	67,69,71,72	0
4	MAN	A	407	11/12	0.55	0.34	-	87,89,90,90	0
5	NAG	B	404	14/15	0.91	0.17	-	51,54,58,63	0

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. LLDF column lists the quality of electron density of the group with respect to its neighbouring residues in protein, DNA or RNA chains. 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	LLDF	B-factors(\AA^2)	Q<0.9
3	PBD	B	402	22/22	0.96	0.10	1.78	17,23,29,32	0
3	PBD	A	402	22/22	0.96	0.10	1.57	17,19,30,32	0
2	ZN	B	401	1/1	1.00	0.10	0.98	19,19,19,19	0
2	ZN	A	401	1/1	1.00	0.07	-1.10	17,17,17,17	0
6	PEG	B	406	7/7	0.89	0.14	-	49,50,53,53	0

6.5 Other polymers [i](#)

There are no such residues in this entry.