Full wwPDB X-ray Structure Validation Report (i)

Mar 10, 2022 – 06:32 pm GMT

PDB ID	:	7QPU
Title	:	Botulinum neurotoxin A5 cell binding domain in complex with GM1b oligosaccharide
Authors	:	Gregory, K.S.; Acharya, K.R.; Liu, S.M.
Deposited on	:	2022-01-05
Resolution	:	2.40 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
https://www.wwpdb.org/validation/2017/XrayValidationReportHelp
with specific help available everywhere you see the (i) symbol.

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

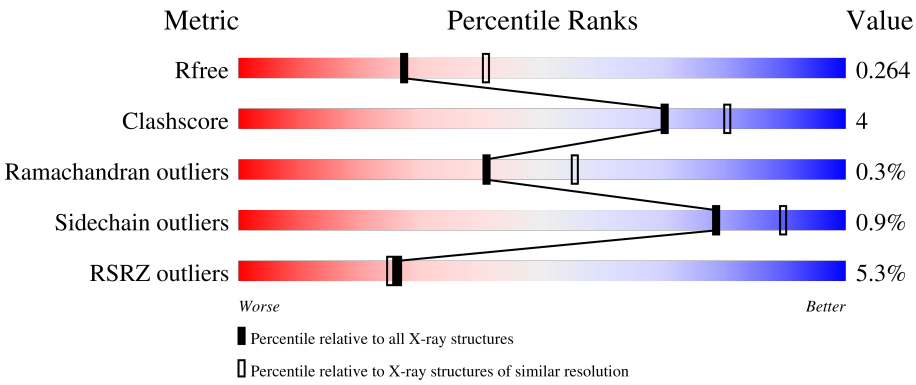
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.27
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0267
CCP4	:	7.1.010 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.27

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 2.40 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	3907 (2.40-2.40)
Clashscore	141614	4398 (2.40-2.40)
Ramachandran outliers	138981	4318 (2.40-2.40)
Sidechain outliers	138945	4319 (2.40-2.40)
RSRZ outliers	127900	3811 (2.40-2.40)

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	433	3%, 80%, 9%, 11%
1	B	433	6%, 72%, 9%, 19%
2	C	2	50%, 50%

2 Entry composition (i)

There are 5 unique types of molecules in this entry. The entry contains 6303 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.

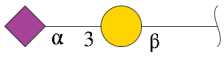
- Molecule 1 is a protein called Botulinum neurotoxin sub-type A5.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	387	3194	2054	538	591	11	0	0	0
1	B	351	2900	1869	481	538	12	0	0	0

There are 14 discrepancies between the modelled and reference sequences:

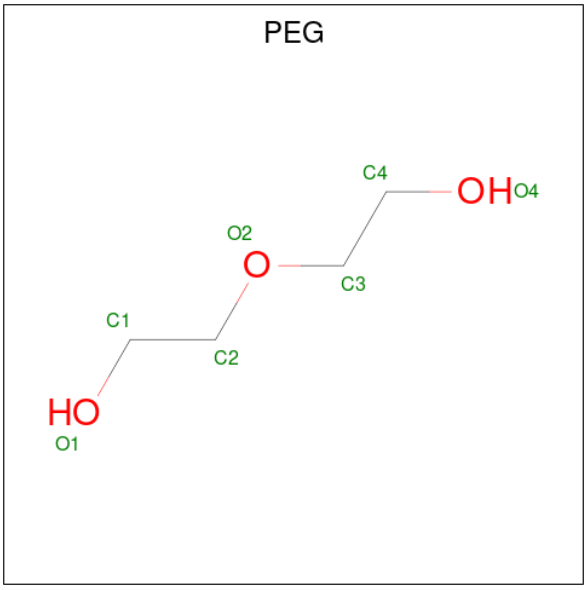
Chain	Residue	Modelled	Actual	Comment	Reference
A	864	MET	-	initiating methionine	UNP C1IPK2
A	865	HIS	-	expression tag	UNP C1IPK2
A	866	HIS	-	expression tag	UNP C1IPK2
A	867	HIS	-	expression tag	UNP C1IPK2
A	868	HIS	-	expression tag	UNP C1IPK2
A	869	HIS	-	expression tag	UNP C1IPK2
A	870	HIS	-	expression tag	UNP C1IPK2
B	864	MET	-	initiating methionine	UNP C1IPK2
B	865	HIS	-	expression tag	UNP C1IPK2
B	866	HIS	-	expression tag	UNP C1IPK2
B	867	HIS	-	expression tag	UNP C1IPK2
B	868	HIS	-	expression tag	UNP C1IPK2
B	869	HIS	-	expression tag	UNP C1IPK2
B	870	HIS	-	expression tag	UNP C1IPK2

- Molecule 2 is an oligosaccharide called N-acetyl-alpha-neuraminic acid-(2-3)-beta-D-galacto pyranose.



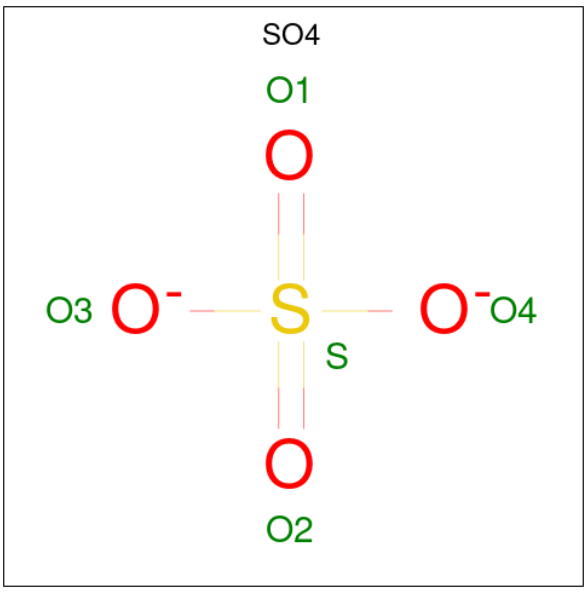
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	Trace
			Total	C	N	O			
2	C	2	32	17	1	14	0	0	0

- Molecule 3 is DI(HYDROXYETHYL)ETHER (three-letter code: PEG) (formula: $C_4H_{10}O_3$).



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

- Molecule 4 is SULFATE ION (three-letter code: SO4) (formula: O_4S).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
			Total	O	S		
4	B	1	5	4	1	0	0

- Molecule 5 is water.

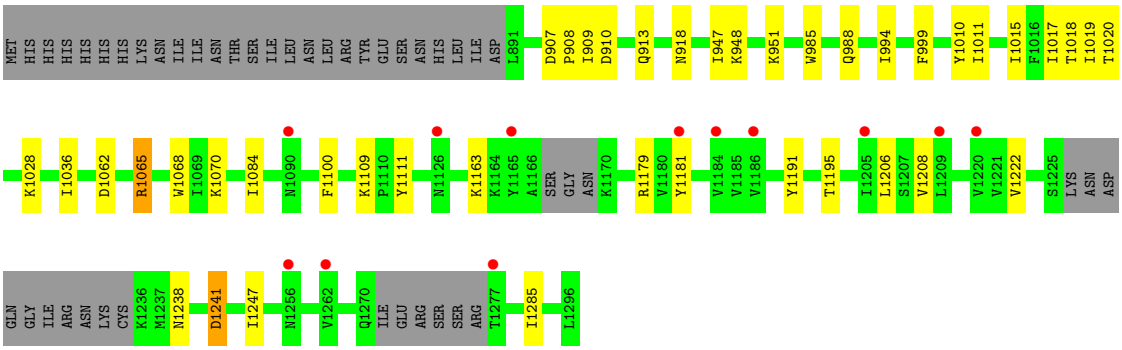
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
5	A	93	93	93	0	0
			Total	O		
5	B	72	72	72	0	0

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.

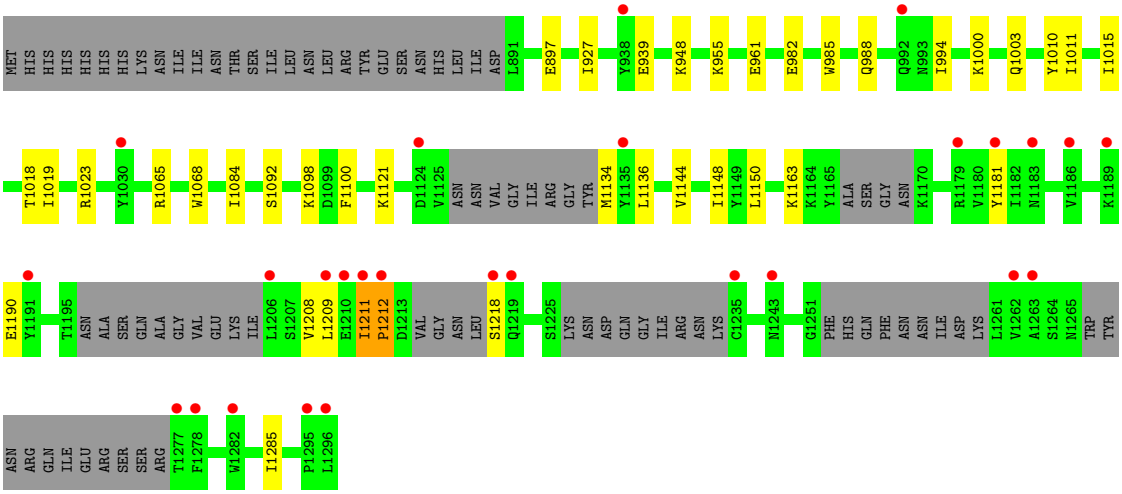
• Molecule 1: Botulinum neurotoxin sub-type A5

Chain A:



• Molecule 1: Botulinum neurotoxin sub-type A5

Chain B:



• Molecule 2: N-acetyl-alpha-neuraminic acid-(2-3)-beta-D-galactopyranose

Chain C:

GAL1
SIA2

4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α, β, γ	44.16Å 129.40Å 78.05Å 90.00° 102.96° 90.00°	Depositor
Resolution (Å)	76.07 – 2.40 76.07 – 2.40	Depositor EDS
% Data completeness (in resolution range)	99.6 (76.07-2.40) 99.7 (76.07-2.40)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	1.32 (at 2.40Å)	Xtriage
Refinement program	REFMAC 1.19.1_4122, PHENIX 1.19.1_4122	Depositor
R, R_{free}	0.219 , 0.260 0.224 , 0.264	Depositor DCC
R_{free} test set	1585 reflections (4.76%)	wwPDB-VP
Wilson B-factor (Å^2)	33.8	Xtriage
Anisotropy	0.170	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	(Not available) , (Not available)	EDS
L-test for twinning[2]	$< \|L\| > = 0.51$, $< L^2 > = 0.34$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.92	EDS
Total number of atoms	6303	wwPDB-VP
Average B, all atoms (Å^2)	38.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 13.47% 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 (i)

5.1 Standard geometry (i)

Bond lengths and bond angles in the following residue types are not validated in this section: PEG, SO4, GAL, SIA

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	0/3264	0.49	0/4414
1	B	0.27	0/2959	0.49	0/3995
All	All	0.27	0/6223	0.49	0/8409

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	3194	0	3153	23	0
1	B	2900	0	2869	21	0
2	C	32	0	28	0	0
3	B	7	0	9	1	0
4	B	5	0	0	0	0
5	A	93	0	0	1	0
5	B	72	0	0	0	0
All	All	6303	0	6059	44	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.

All (44) 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:961:GLU:H	3:B:1301:PEG:H22	1.30	0.93
1:B:1211:ILE:HB	1:B:1212:PRO:HD3	1.65	0.77
1:B:1144:VAL:HG22	1:B:1150:LEU:HG	1.75	0.67
1:B:1092:SER:O	1:B:1098:LYS:NZ	2.32	0.61
1:B:1208:VAL:HG12	1:B:1209:LEU:H	1.66	0.60
1:B:955:LYS:HD2	1:B:1148:ILE:HD11	1.85	0.58
1:A:1020:THR:HG22	1:A:1028:LYS:HB2	1.87	0.57
1:A:1191:TYR:HB2	1:A:1208:VAL:HG12	1.86	0.57
1:A:985:TRP:CD2	1:A:1019:ILE:HG21	2.41	0.56
1:B:897:GLU:HB2	1:B:927:ILE:HB	1.88	0.55
1:A:1179:ARG:HG2	1:A:1222:VAL:HG22	1.89	0.53
1:B:948:LYS:HB3	1:B:1068:TRP:HB2	1.91	0.53
1:A:1241:ASP:HB3	1:A:1247:ILE:HD11	1.92	0.52
1:A:1163:LYS:HB2	1:A:1181:TYR:HB2	1.93	0.51
1:B:1163:LYS:HB2	1:B:1181:TYR:HB2	1.93	0.51
1:B:1010:TYR:HD1	1:B:1015:ILE:HG21	1.78	0.49
1:A:999:PHE:HD1	1:A:1036:ILE:HG21	1.78	0.49
1:A:951:LYS:HD3	1:A:1011:ILE:HG21	1.95	0.48
1:A:988:GLN:HG2	1:A:994:ILE:HG12	1.95	0.48
1:B:982:GLU:HG2	1:B:1000:LYS:HG2	1.96	0.48
1:B:1003:GLN:HA	1:B:1011:ILE:HD11	1.94	0.47
1:A:948:LYS:HB3	1:A:1068:TRP:HB2	1.96	0.47
1:B:988:GLN:HG2	1:B:994:ILE:HG22	1.96	0.47
1:A:913:GLN:HG2	1:A:1070:LYS:HD3	1.96	0.47
1:A:1100:PHE:HB2	1:A:1285:ILE:HG12	1.97	0.46
1:B:985:TRP:CD2	1:B:1019:ILE:HG21	2.50	0.46
1:B:1018:THR:HG21	1:B:1084:ILE:HG12	1.98	0.45
1:A:947:ILE:HG22	1:A:1017:ILE:HD13	1.99	0.45
1:A:1062:ASP:HB3	1:A:1065:ARG:HB2	1.98	0.45
1:A:907:ASP:HB3	1:A:910:ASP:O	2.17	0.44
1:A:918:ASN:ND2	1:A:1062:ASP:O	2.36	0.44
1:A:985:TRP:CE2	1:A:1019:ILE:HG21	2.52	0.44
1:B:1121:LYS:HD3	1:B:1136:LEU:HB3	2.00	0.44
1:A:1010:TYR:HD1	1:A:1015:ILE:HG21	1.83	0.43
1:A:908:PRO:HG2	1:A:909:ILE:HD12	2.00	0.43
1:B:1211:ILE:CB	1:B:1212:PRO:HD3	2.43	0.43
1:B:1100:PHE:HB2	1:B:1285:ILE:HG12	2.00	0.43
1:A:1109:LYS:HD3	1:A:1111:TYR:OH	2.18	0.43
1:B:1134:MET:SD	1:B:1209:LEU:HD21	2.60	0.41
1:B:939:GLU:HB2	1:B:1023:ARG:HD2	2.02	0.41

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:B:985:TRP:CE2	1:B:1019:ILE:HG21	2.55	0.41
1:A:1018:THR:HG21	1:A:1084:ILE:HG12	2.02	0.41
1:A:1195:THR:HB	1:A:1206:LEU:HD23	2.01	0.41
1:A:1020:THR:HG23	5:A:1324:HOH:O	2.20	0.41

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	379/433 (88%)	365 (96%)	14 (4%)	0	100	100
1	B	335/433 (77%)	318 (95%)	15 (4%)	2 (1%)	25	36
All	All	714/866 (82%)	683 (96%)	29 (4%)	2 (0%)	41	55

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	B	1211	ILE
1	B	1212	PRO

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.

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	A	357/401 (89%)	354 (99%)	3 (1%)	81 91
1	B	328/401 (82%)	325 (99%)	3 (1%)	78 90
All	All	685/802 (85%)	679 (99%)	6 (1%)	78 90

All (6) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	1065	ARG
1	A	1238	ASN
1	A	1241	ASP
1	B	1065	ARG
1	B	1190	GLU
1	B	1218	SER

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

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)

2 monosaccharides 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	$\#\|Z\| > 2$
2	GAL	C	1	2	12,12,12	0.56	0	17,17,17	0.76	0
2	SIA	C	2	2	17,20,21	1.07	1 (5%)	21,28,31	1.34	2 (9%)

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
2	GAL	C	1	2	-	0/2/22/22	0/1/1/1
2	SIA	C	2	2	-	6/14/34/38	0/1/1/1

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	C	2	SIA	C7-C6	2.32	1.55	1.53

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	C	2	SIA	C6-O6-C2	4.10	120.11	111.34
2	C	2	SIA	C4-C3-C2	2.04	113.47	109.81

There are no chirality outliers.

All (6) torsion outliers are listed below:

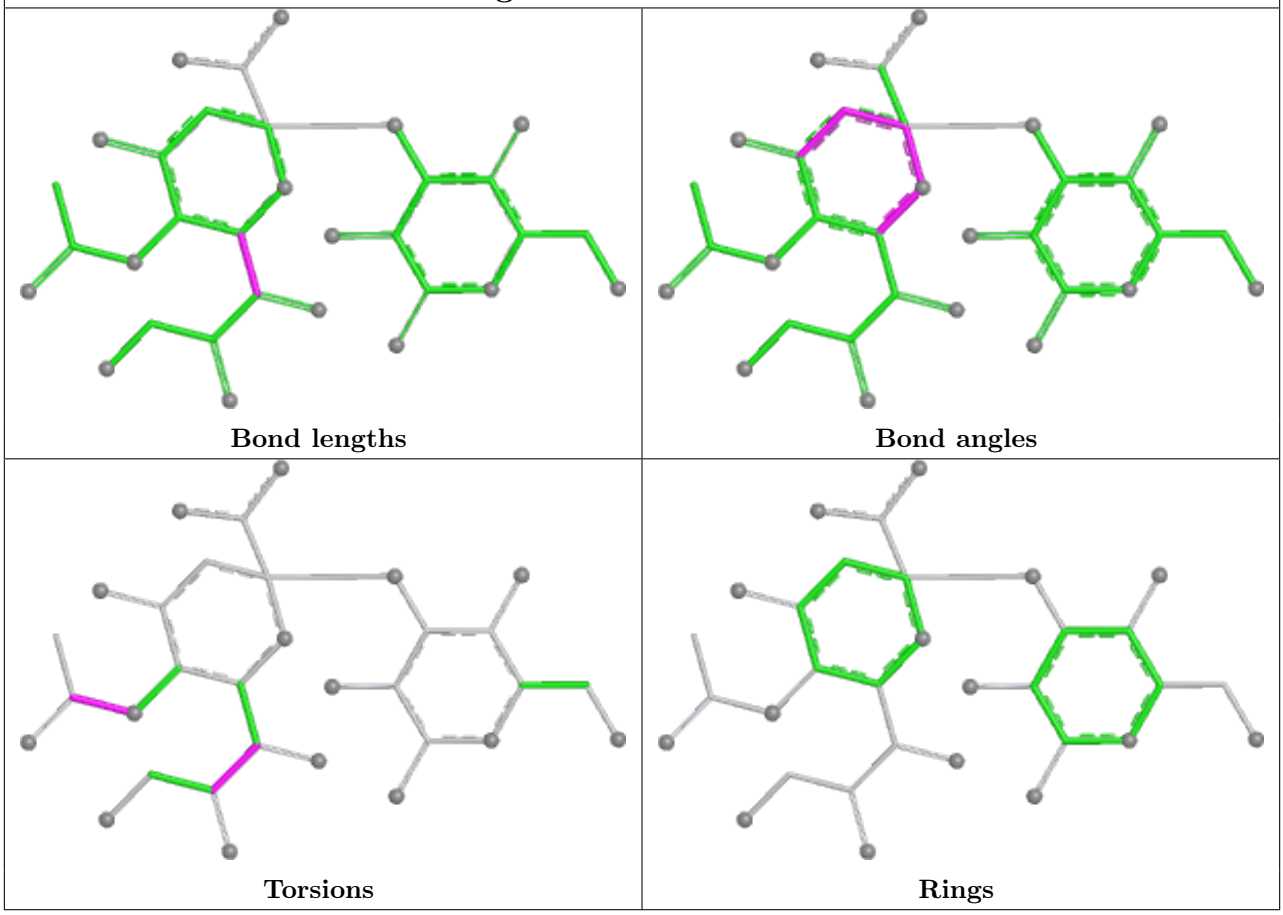
Mol	Chain	Res	Type	Atoms
2	C	2	SIA	C6-C7-C8-C9
2	C	2	SIA	C6-C7-C8-O8
2	C	2	SIA	O7-C7-C8-C9
2	C	2	SIA	O7-C7-C8-O8
2	C	2	SIA	C11-C10-N5-C5
2	C	2	SIA	O10-C10-N5-C5

There are no ring outliers.

No monomer is involved in short contacts.

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for oligosaccharide.

Oligosaccharide Chain C



5.6 Ligand geometry (i)

2 ligands 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	$\#\|Z\| > 2$
3	PEG	B	1301	-	6,6,6	0.12	0	5,5,5	0.08	0
4	SO4	B	1302	-	4,4,4	0.15	0	6,6,6	0.11	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	PEG	B	1301	-	-	2/4/4/4	-

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (2) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	B	1301	PEG	C4-C3-O2-C2
3	B	1301	PEG	O2-C3-C4-O4

There are no ring outliers.

1 monomer is involved in 1 short contact:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	B	1301	PEG	1	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	387/433 (89%)	0.45	12 (3%)	49	47	18, 35, 60, 76	0
1	B	351/433 (81%)	0.52	27 (7%)	13	12	20, 36, 69, 85	0
All	All	738/866 (85%)	0.48	39 (5%)	26	25	18, 35, 65, 85	0

All (39) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	B	1210	GLU	5.6
1	B	1296	LEU	4.6
1	B	1235	CYS	4.0
1	B	1191	TYR	4.0
1	B	1179	ARG	3.7
1	B	1218	SER	3.6
1	A	1256	ASN	3.5
1	B	1209	LEU	3.5
1	B	938	TYR	3.3
1	B	1295	PRO	3.3
1	A	1209	LEU	3.2
1	A	1220	VAL	3.1
1	B	1262	VAL	3.1
1	B	1277	THR	3.1
1	B	1124	ASP	2.9
1	B	1263	ALA	2.9
1	B	1206	LEU	2.9
1	B	1186	VAL	2.8
1	A	1184	VAL	2.7
1	A	1277	THR	2.6
1	A	1126	ASN	2.6
1	A	1181	TYR	2.5
1	A	1186	VAL	2.5
1	B	1181	TYR	2.5

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Mol	Chain	Res	Type	RSRZ
1	B	1135	TYR	2.3
1	B	1211	ILE	2.3
1	B	992	GLN	2.3
1	B	1282	TRP	2.2
1	A	1165	TYR	2.2
1	B	1030	TYR	2.2
1	A	1205	ILE	2.2
1	B	1243	ASN	2.2
1	B	1183	ASN	2.1
1	A	1090	ASN	2.1
1	B	1278	PHE	2.1
1	B	1212	PRO	2.1
1	A	1262	VAL	2.0
1	B	1189	LYS	2.0
1	B	1219	GLN	2.0

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)

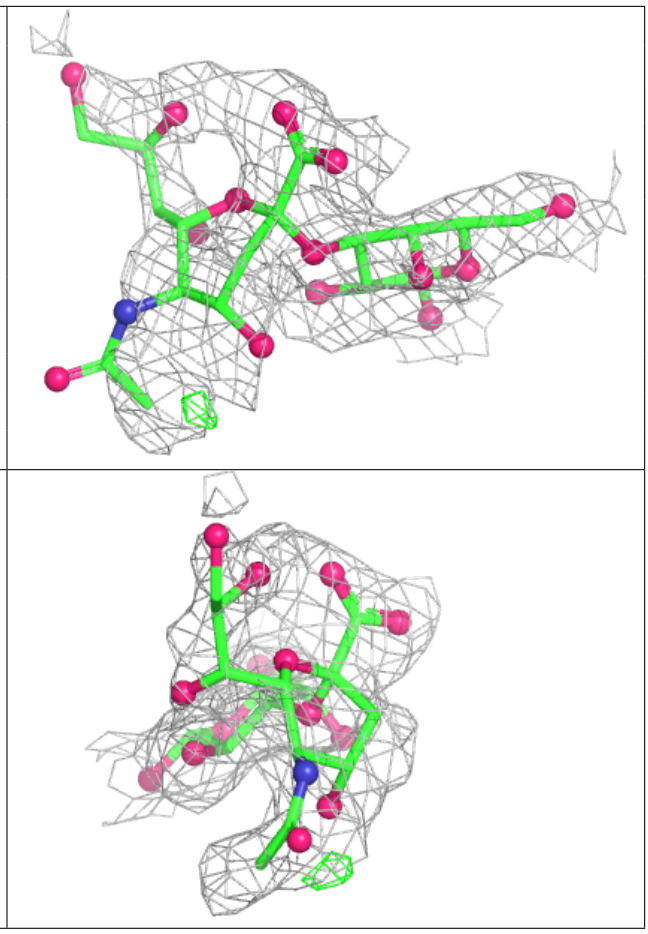
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95th percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists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

Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å^2)	Q<0.9
2	SIA	C	2	20/21	0.60	0.36	51,74,88,92	0
2	GAL	C	1	12/12	0.69	0.26	56,64,72,73	0

The following is a graphical depiction of the model fit to experimental electron density for oligosaccharide. Each fit is shown from different orientation to approximate a three-dimensional view.

Electron density around Chain C:

$2mF_o$-DF_c (at 0.7 rmsd) in gray
mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)



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, 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
3	PEG	B	1301	7/7	0.77	0.25	39,45,49,61	0
4	SO4	B	1302	5/5	0.92	0.16	33,35,49,56	0

6.5 Other polymers (i)

There are no such residues in this entry.