W O R L D W I D E

wwPDB

P R O T E I N D A T A B A N K

wwPDB EM Validation Summary Report (i)

Mar 3, 2025 – 12:23 pm GMT

PDB ID	:	9F2Y
EMDB ID	:	EMD-50166
Title	:	Focused refinement of SV2B-LD-BoNT/A1 at pH 5.5
Authors	:	Khanppnavar, B.; Leka, O.; Korkhov, V.; Kammerer, R.
Deposited on	:	2024-04-24
Resolution	:	4.39 Å(reported)

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

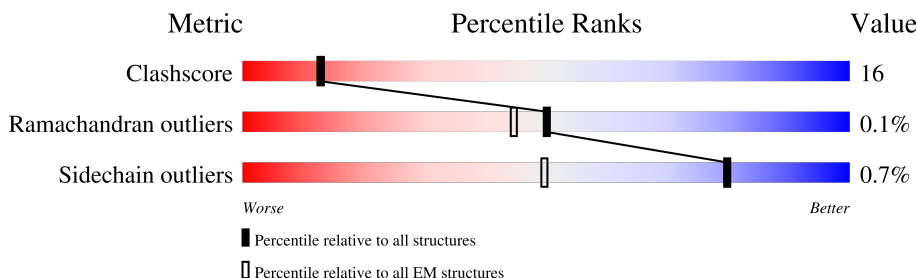|		
EMDB validation analysis	:	FAILED
Mogul	:	1.8.4, CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20231227.v01 (using entries in the PDB archive December 27th 2023)
MapQ	:	FAILED
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.41

1   Overall quality at a glance ⓘ

The following experimental techniques were used to determine the structure:
ELECTRON MICROSCOPY

The reported resolution of this entry is 4.39 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	Percentile Ranks	Value
Clashscore		16
Ramachandran outliers		0.1%
Sidechain outliers		0.7%

Worse   *Better*

▮ Percentile relative to all structures

▯ Percentile relative to all EM structures

Metric	Whole archive (#Entries)	EM structures (#Entries)
Clashscore	210492	15764
Ramachandran outliers	207382	16835
Sidechain outliers	206894	16415

The table below summarises the geometric issues observed across the polymeric chains and their fit to the map. The red, orange, yellow and green segments of the 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%

Mol	Chain	Length	Quality of chain
1	A	683	9% 5% 86%
2	B	1329	60% 33% 7%
3	C	3	67% 33%

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
4	NAG	A	702	X	-	-	-

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2   Entry composition   (i)

There are 4 unique types of molecules in this entry. The entry contains 10994 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, 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 Synaptic vesicle glycoprotein 2B.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	A	97	833	542	130	155	6	0	0

- Molecule 2 is a protein called Botulinum neurotoxin type A.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
2	B	1239	10094	6490	1658	1914	32	0	0

There are 39 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	-16	MET	-	initiating methionine	UNP P0DPI0
B	-15	ARG	-	expression tag	UNP P0DPI0
B	-14	GLY	-	expression tag	UNP P0DPI0
B	-13	SER	-	expression tag	UNP P0DPI0
B	-12	HIS	-	expression tag	UNP P0DPI0
B	-11	HIS	-	expression tag	UNP P0DPI0
B	-10	HIS	-	expression tag	UNP P0DPI0
B	-9	HIS	-	expression tag	UNP P0DPI0
B	-8	HIS	-	expression tag	UNP P0DPI0
B	-7	HIS	-	expression tag	UNP P0DPI0
B	-6	GLY	-	expression tag	UNP P0DPI0
B	-5	SER	-	expression tag	UNP P0DPI0
B	-4	LEU	-	expression tag	UNP P0DPI0
B	-3	VAL	-	expression tag	UNP P0DPI0
B	-2	PRO	-	expression tag	UNP P0DPI0
B	-1	ARG	-	expression tag	UNP P0DPI0
B	0	GLY	-	expression tag	UNP P0DPI0
B	1	SER	-	expression tag	UNP P0DPI0
B	27	ALA	VAL	variant	UNP P0DPI0
B	224	GLN	GLU	engineered mutation	UNP P0DPI0
B	363	ALA	ARG	engineered mutation	UNP P0DPI0

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Chain	Residue	Modelled	Actual	Comment	Reference
B	366	PHE	TYR	engineered mutation	UNP P0DPI0
B	1158	ALA	THR	conflict	UNP P0DPI0
B	1297	VAL	-	expression tag	UNP P0DPI0
B	1298	PRO	-	expression tag	UNP P0DPI0
B	1299	PRO	-	expression tag	UNP P0DPI0
B	1300	THR	-	expression tag	UNP P0DPI0
B	1301	PRO	-	expression tag	UNP P0DPI0
B	1302	GLY	-	expression tag	UNP P0DPI0
B	1303	SER	-	expression tag	UNP P0DPI0
B	1304	ALA	-	expression tag	UNP P0DPI0
B	1305	TRP	-	expression tag	UNP P0DPI0
B	1306	SER	-	expression tag	UNP P0DPI0
B	1307	HIS	-	expression tag	UNP P0DPI0
B	1308	PRO	-	expression tag	UNP P0DPI0
B	1309	GLN	-	expression tag	UNP P0DPI0
B	1310	PHE	-	expression tag	UNP P0DPI0
B	1311	GLU	-	expression tag	UNP P0DPI0
B	1312	LYS	-	expression tag	UNP P0DPI0

- Molecule 3 is an oligosaccharide called beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose.
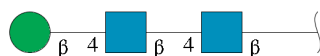
Mol	Chain	Residues	Atoms				AltConf	Trace
3	C	3	Total 39	C 22	N 2	O 15	0	0

- Molecule 4 is 2-acetamido-2-deoxy-beta-D-glucopyranose (three-letter code: NAG) (formula: $C_8H_{15}NO_6$) (labeled as "Ligand of Interest" by depositor).

NAG

| Mol	Chain	Residues	Atoms				AltConf
4	A	1	Total	C	N	O	0
			14	8	1	5	
4	A	1	Total	C	N	O	0
			14	8	1	5	

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. 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. 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: Synaptic vesicle glycoprotein 2B

Chain A: 9% 5% 86%
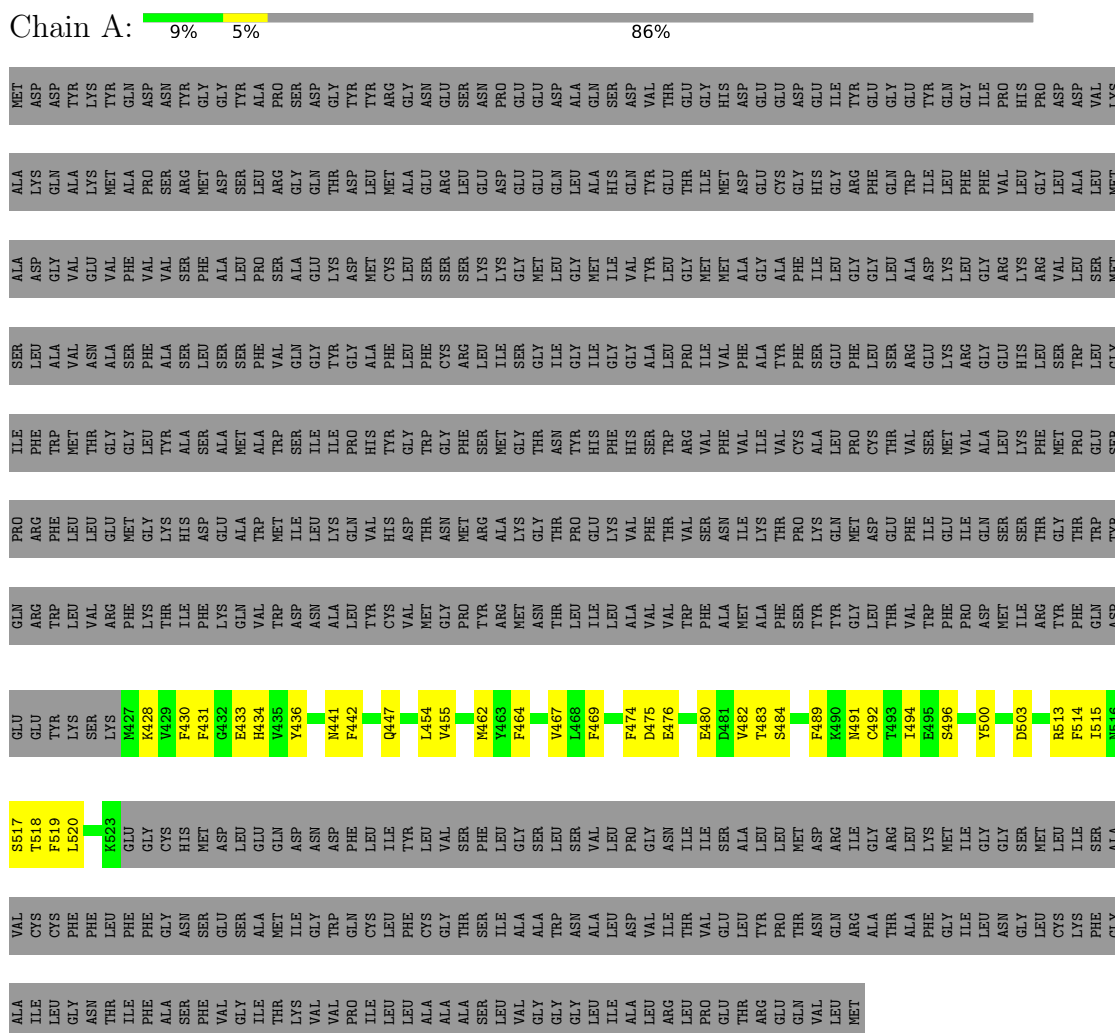
• Molecule 2: Botulinum neurotoxin type A

Chain B: 60% 33% 7%
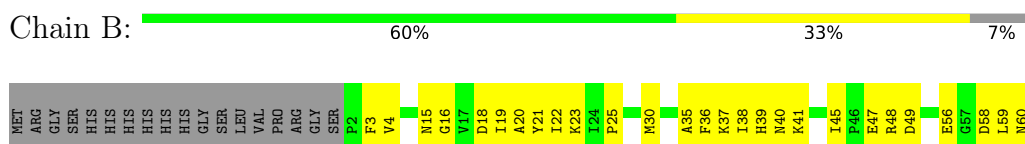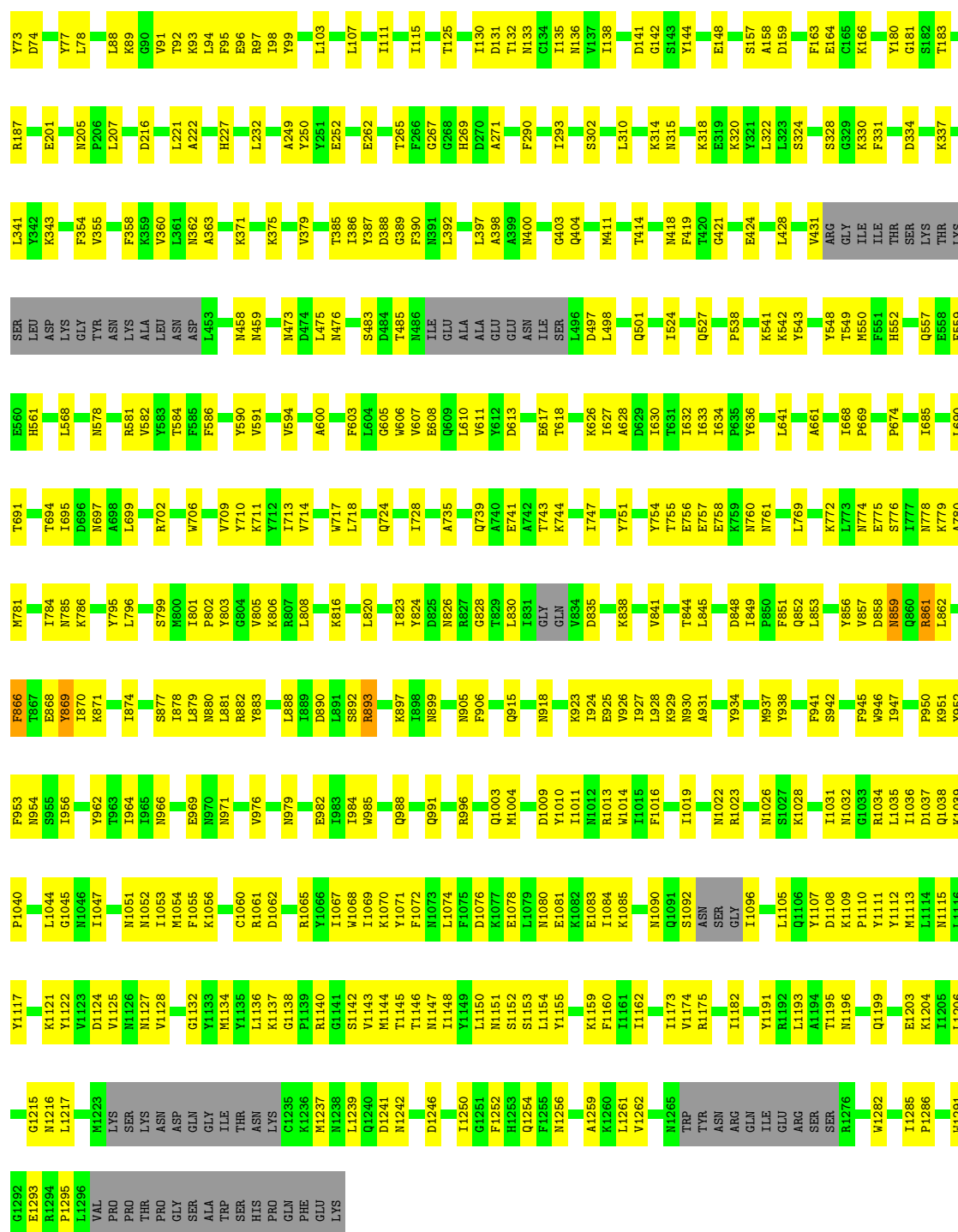
● Molecule 3: beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-ac
etamido-2-deoxy-beta-D-glucopyranose

4 Experimental information ⓘ

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	187001	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	TFS KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose $(e^-/Å^2)$	50	Depositor
Minimum defocus (nm)	600	Depositor
Maximum defocus (nm)	2400	Depositor
Magnification	Not provided	
Image detector	GATAN K3 BIOQUANTUM (6k x 4k)	Depositor

5 Model quality ⓘ

5.1 Standard geometry ⓘ

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

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	0.30	0/857	0.48	0/1154
2	B	0.29	0/10307	0.51	0/13952
All	All	0.29	0/11164	0.51	0/15106

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
2	B	0	1

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
2	B	861	ARG	Sidechain

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.

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	833	0	769	29	0
2	B	10094	0	9983	334	0
3	C	39	0	34	3	0
4	A	28	0	26	3	0
All	All	10994	0	10812	356	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 16.

The worst 5 of 356 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:491:ASN:ND2	4:A:701:NAG:C1	1.72	1.50
1:A:517:SER:O	2:B:1143:VAL:HG23	1.46	1.13
2:B:934:TYR:HB2	2:B:937:MET:HB3	1.58	0.85
2:B:953:PHE:HE2	3:C:1:NAG:H62	1.41	0.84
2:B:953:PHE:CE2	3:C:1:NAG:H62	2.14	0.82

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 PDB entries followed by that with respect to all EM entries.

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	95/683 (14%)	88 (93%)	7 (7%)	0	100	100
2	B	1225/1329 (92%)	1159 (95%)	65 (5%)	1 (0%)	48	83
All	All	1320/2012 (66%)	1247 (94%)	72 (6%)	1 (0%)	50	83

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
2	B	859	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 PDB entries followed by that with respect to all EM entries.

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	93/580 (16%)	90 (97%)	3 (3%)	34	55
2	B	1127/1204 (94%)	1122 (100%)	5 (0%)	89	91
All	All	1220/1784 (68%)	1212 (99%)	8 (1%)	80	87

5 of 8 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	B	1085	LYS
2	B	893	ARG
2	B	866	PHE
2	B	626	LYS
2	B	869	TYR

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 17 such sidechains are listed below:

Mol	Chain	Res	Type
2	B	1032	ASN
2	B	1176	ASN
2	B	557	GLN
2	B	697	ASN
2	B	739	GLN

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 ⓘ

3 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	$\#|Z| > 2$
3	NAG	C	1	1,3	14,14,15	0.38	0	17,19,21	0.58	0
3	NAG	C	2	3	14,14,15	0.21	0	17,19,21	0.41	0
3	BMA	C	3	3	11,11,12	0.57	0	15,15,17	0.84	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	NAG	C	1	1,3	-	3/6/23/26	0/1/1/1
3	NAG	C	2	3	-	0/6/23/26	0/1/1/1
3	BMA	C	3	3	-	0/2/19/22	0/1/1/1

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (3) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	C	1	NAG	O5-C5-C6-O6
3	C	1	NAG	C4-C5-C6-O6
3	C	1	NAG	C3-C2-N2-C7

There are no ring outliers.

1 monomer is involved in 3 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	C	1	NAG	3	0

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

Bond lengths

Bond angles

Torsions

Rings
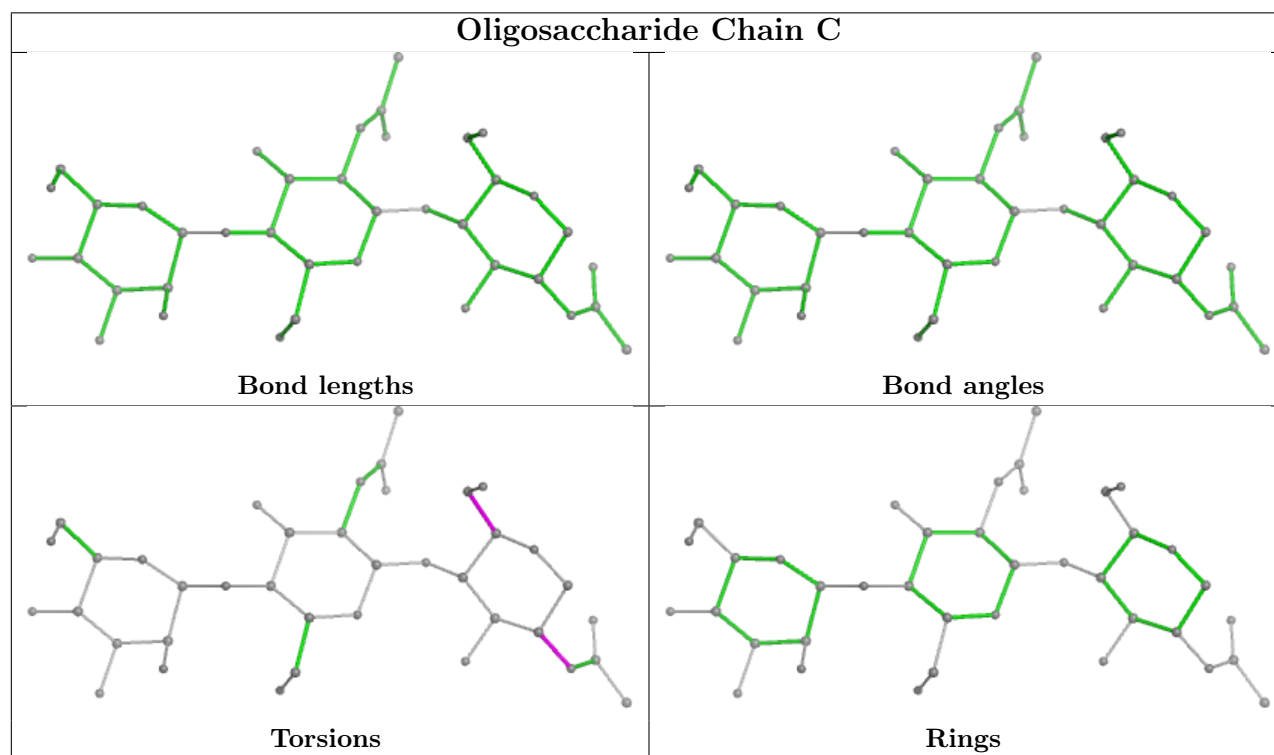
5.6 Ligand geometry ⓘ

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	$\#|Z| > 2$
4	NAG	A	701	-	14,14,15	0.39	0	17,19,21	0.61	0
4	NAG	A	702	1	14,14,15	0.43	0	17,19,21	0.64	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	701	-	-	4/6/23/26	0/1/1/1
4	NAG	A	702	1	1/1/5/7	2/6/23/26	0/1/1/1

There are no bond length outliers.

There are no bond angle outliers.

All (1) chirality outliers are listed below:

Mol	Chain	Res	Type	Atom
4	A	702	NAG	C1

5 of 6 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
4	A	701	NAG	O5-C5-C6-O6
4	A	701	NAG	C8-C7-N2-C2
4	A	701	NAG	C4-C5-C6-O6
4	A	701	NAG	O7-C7-N2-C2
4	A	702	NAG	C1-C2-N2-C7

There are no ring outliers.

2 monomers are involved in 3 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	A	701	NAG	2	0
4	A	702	NAG	1	0

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less then 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.

Ligand NAG A 701

Bond lengths

Bond angles

Torsions

Rings

Ligand NAG A 702

Bond lengths

Bond angles

Torsions

Rings
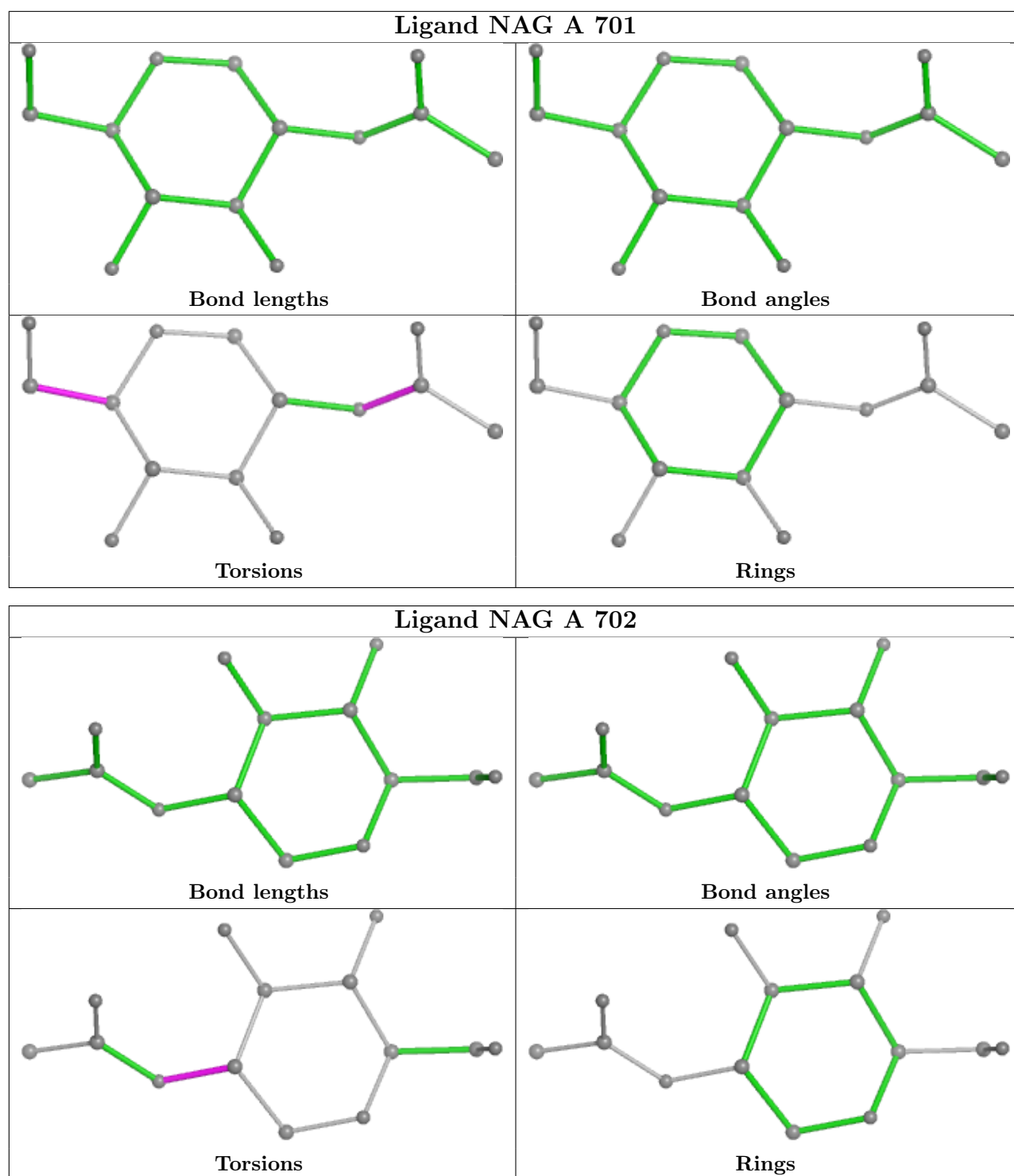
5.7 Other polymers ⓘ

There are no such residues in this entry.

5.8   Polymer linkage issues (i)

There are no chain breaks in this entry.