



## wwPDB EM Validation Summary Report ⓘ

Nov 26, 2024 – 10:07 AM JST

PDB ID : 8KEI  
EMDB ID : EMD-37159  
Title : Cryo-EM structure of NADPH oxidase 2 in complex with p22phox and EROS  
Authors : Liang, S.Y.; Liu, A.J.; Liu, Y.Z.; Ye, R.D.  
Deposited on : 2023-08-11  
Resolution : 3.56 Å(reported)

This is a wwPDB EM Validation Summary Report for a publicly released PDB entry.

We welcome your comments at [validation@mail.wwpdb.org](mailto:validation@mail.wwpdb.org)

A user guide is available at

<https://www.wwpdb.org/validation/2017/EMValidationReportHelp>

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>.

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

EMDB validation analysis	: <b>FAILED</b>
Mogul	: 1.8.5 (274361), 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	: <b>FAILED</b>
Ideal geometry (proteins)	: Engh & Huber (2001)
Ideal geometry (DNA, RNA)	: Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	: 2.40

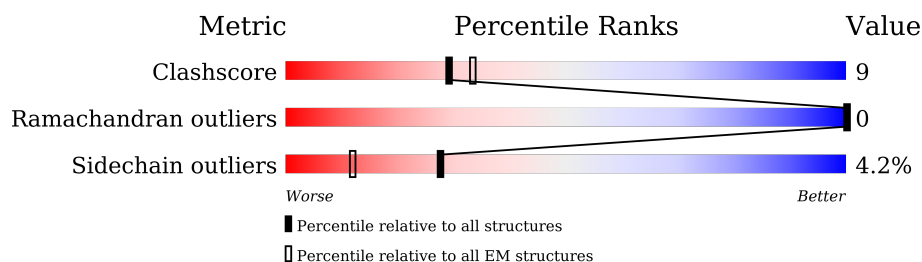
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*ELECTRON MICROSCOPY*

The reported resolution of this entry is 3.56 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)	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  $\geq 3$ , 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  $\leq 5\%$ .

Mol	Chain	Length	Quality of chain
1	A	133	74% 25% .
2	B	565	73% 20% . 5%
3	C	224	79% 20% .
4	D	164	79% 21%
5	E	217	75% 24%
6	F	3	100%
6	G	3	67% 33%

## 2 Entry composition

There are 10 unique types of molecules in this entry. The entry contains 10209 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 Cytochrome b-245 light chain.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	133	Total	C	N	O	S	0	0
			1035	684	173	173	5		

- Molecule 2 is a protein called Cytochrome b-245 heavy chain.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	B	534	Total	C	N	O	S	0	0
			4318	2826	730	737	25		

- Molecule 3 is a protein called monoclonal antibody 7G5 heavy chain.

Mol	Chain	Residues	Atoms					AltConf	Trace
3	C	224	Total	C	N	O	S	0	0
			1648	1038	273	328	9		

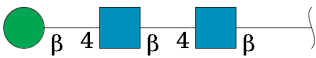
- Molecule 4 is a protein called Cytochrome b-245 chaperone 1.

Mol	Chain	Residues	Atoms					AltConf	Trace
4	D	164	Total	C	N	O	S	0	0
			1304	848	221	231	4		

- Molecule 5 is a protein called monoclonal antibody 7G5 light chain.

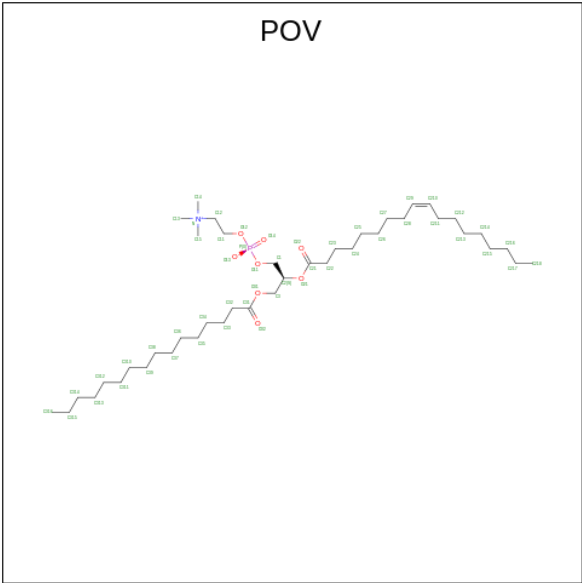
Mol	Chain	Residues	Atoms					AltConf	Trace
5	E	217	Total	C	N	O	S	0	0
			1652	1033	278	335	6		

- Molecule 6 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.



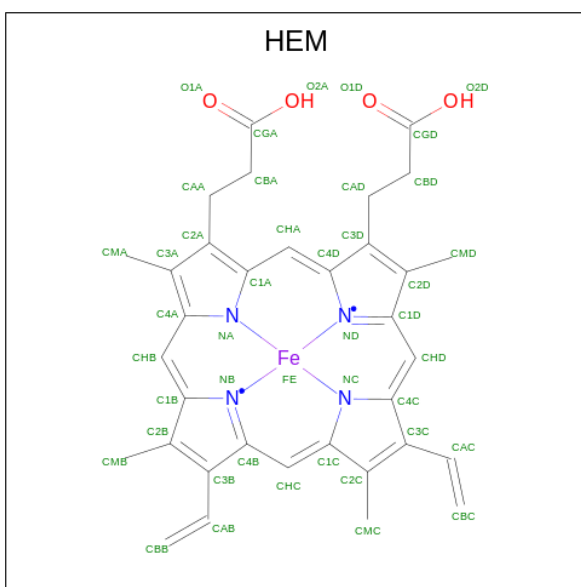
Mol	Chain	Residues	Atoms				AltConf	Trace
6	F	3	Total	C	N	O	0	0
			39	22	2	15		
6	G	3	Total	C	N	O	0	0
			39	22	2	15		

- Molecule 7 is (2S)-3-(hexadecanoyloxy)-2-[(9Z)-octadec-9-enoyloxy]propyl 2-(trimethylamm onio)ethyl phosphate (three-letter code: POV) (formula: C<sub>42</sub>H<sub>82</sub>NO<sub>8</sub>P) (labeled as "Ligand of Interest" by depositor).



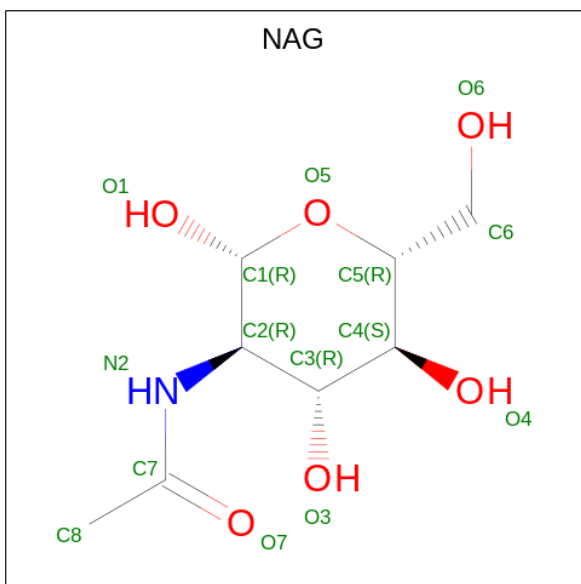
Mol	Chain	Residues	Atoms				AltConf
7	A	1	Total	C	O	P	0
			26	19	6	1	

- Molecule 8 is PROTOPORPHYRIN IX CONTAINING FE (three-letter code: HEM) (for- mula: C<sub>34</sub>H<sub>32</sub>FeN<sub>4</sub>O<sub>4</sub>) (labeled as "Ligand of Interest" by depositor).



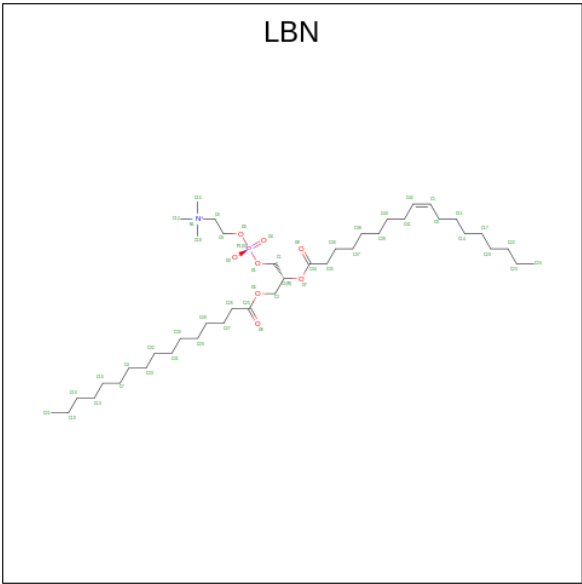
Mol	Chain	Residues	Atoms					AltConf
8	B	1	Total	C	Fe	N	O	0
			43	34	1	4	4	
8	B	1	Total	C	Fe	N	O	0
			43	34	1	4	4	

- Molecule 9 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).



Mol	Chain	Residues	Atoms				AltConf
9	B	1	Total	C	N	O	0
			14	8	1	5	

- Molecule 10 is 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (three-letter code: LBN) (formula: C<sub>42</sub>H<sub>82</sub>NO<sub>8</sub>P).



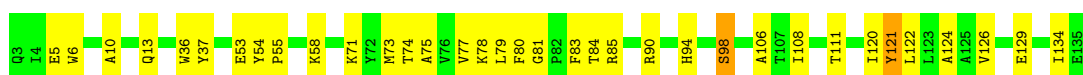
Mol	Chain	Residues	Atoms					AltConf
			Total	C	N	O	P	
10	B	1	48	38	1	8	1	0

### 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. 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.

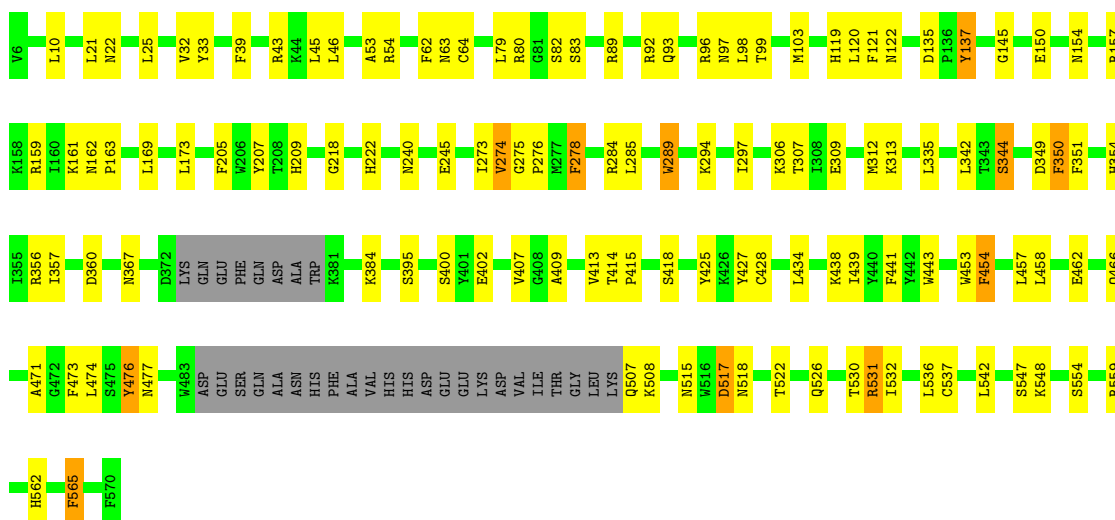
- Molecule 1: Cytochrome b-245 light chain

Chain A: 




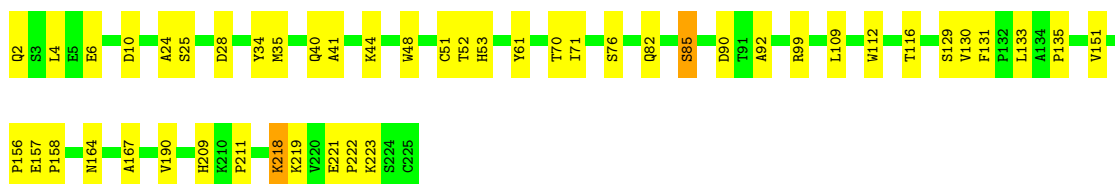
- Molecule 2: Cytochrome b-245 heavy chain

Chain B: 




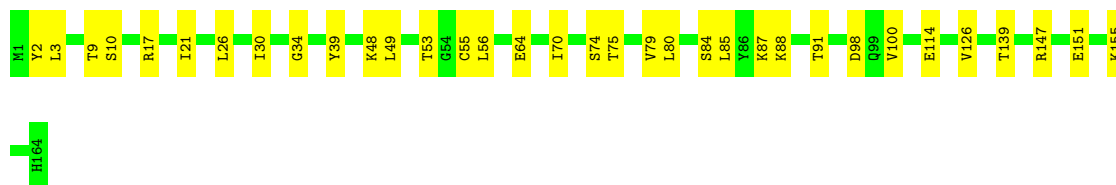
- Molecule 3: monoclonal antibody 7G5 heavy chain

Chain C: 



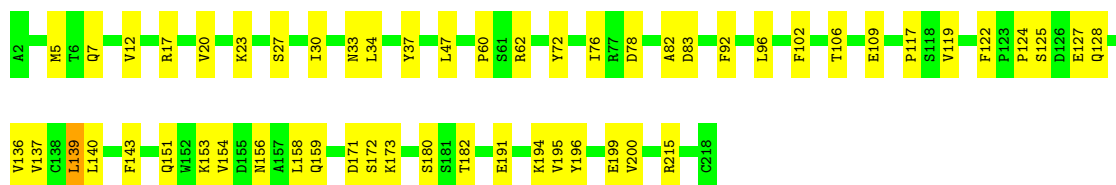
- Molecule 4: Cytochrome b-245 chaperone 1

Chain D:  79% 21%



- Molecule 5: monoclonal antibody 7G5 light chain

Chain E:  75% 24%



- Molecule 6: beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose

Chain F:  100%



- Molecule 6: beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose

Chain G:  67% 33%



## 4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	131926	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ( $e^-/\text{\AA}^2$ )	52	Depositor
Minimum defocus (nm)	1200	Depositor
Maximum defocus (nm)	2500	Depositor
Magnification	Not provided	
Image detector	GATAN K3 BIOQUANTUM (6k x 4k)	Depositor

## 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: POV, NAG, BMA, HEM, LBN

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	0.26	0/1062	0.50	0/1444
2	B	0.31	0/4439	0.53	0/6024
3	C	0.27	0/1690	0.53	0/2305
4	D	0.26	0/1332	0.50	0/1801
5	E	0.26	0/1685	0.51	0/2288
All	All	0.29	0/10208	0.52	0/13862

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	1035	0	1062	25	0
2	B	4318	0	4337	72	0
3	C	1648	0	1603	31	0
4	D	1304	0	1338	23	0
5	E	1652	0	1610	36	0
6	F	39	0	34	0	0
6	G	39	0	34	0	0
7	A	26	0	35	1	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
8	B	86	0	60	5	0
9	B	14	0	13	0	0
10	B	48	0	0	0	0
All	All	10209	0	10126	184	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 9.

The worst 5 of 184 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)
2:B:476:TYR:HH	2:B:507:GLN:N	1.65	0.93
3:C:133:LEU:HD11	5:E:122:PHE:HB3	1.64	0.80
2:B:559:ARG:O	2:B:559:ARG:NH1	2.15	0.76
2:B:413:VAL:HG22	2:B:454:PHE:HE2	1.51	0.75
1:A:84:THR:HG22	1:A:85:ARG:H	1.51	0.74

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 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	131/133 (98%)	123 (94%)	8 (6%)	0	100	100
2	B	528/565 (94%)	492 (93%)	36 (7%)	0	100	100
3	C	222/224 (99%)	208 (94%)	14 (6%)	0	100	100
4	D	162/164 (99%)	157 (97%)	5 (3%)	0	100	100
5	E	215/217 (99%)	203 (94%)	12 (6%)	0	100	100
All	All	1258/1303 (96%)	1183 (94%)	75 (6%)	0	100	100

There are no Ramachandran outliers to report.

### 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 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	102/102 (100%)	97 (95%)	5 (5%)	21	50
2	B	468/495 (94%)	440 (94%)	28 (6%)	16	44
3	C	185/185 (100%)	180 (97%)	5 (3%)	40	65
4	D	141/141 (100%)	139 (99%)	2 (1%)	62	80
5	E	184/184 (100%)	179 (97%)	5 (3%)	40	65
All	All	1080/1107 (98%)	1035 (96%)	45 (4%)	27	54

5 of 45 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	B	517	ASP
3	C	85	SER
2	B	518	ASN
2	B	565	PHE
3	C	221	GLU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 6 such sidechains are listed below:

Mol	Chain	Res	Type
2	B	477	ASN
2	B	518	ASN
2	B	553	ASN
2	B	338	HIS
2	B	22	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](#)

There are no non-standard protein/DNA/RNA residues in this entry.

## 5.5 Carbohydrates [i](#)

6 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	$\# Z  > 2$
6	NAG	F	1	2,6	14,14,15	0.43	0	17,19,21	0.50	0
6	NAG	F	2	6	14,14,15	0.20	0	17,19,21	0.38	0
6	BMA	F	3	6	11,11,12	0.62	0	15,15,17	0.71	0
6	NAG	G	1	2,6	14,14,15	0.46	0	17,19,21	0.64	0
6	NAG	G	2	6	14,14,15	0.24	0	17,19,21	0.68	0
6	BMA	G	3	6	11,11,12	1.83	2 (18%)	15,15,17	1.85	4 (26%)

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
6	NAG	F	1	2,6	-	0/6/23/26	0/1/1/1
6	NAG	F	2	6	-	0/6/23/26	0/1/1/1
6	BMA	F	3	6	-	0/2/19/22	0/1/1/1
6	NAG	G	1	2,6	-	2/6/23/26	0/1/1/1
6	NAG	G	2	6	-	0/6/23/26	0/1/1/1
6	BMA	G	3	6	-	2/2/19/22	0/1/1/1

All (2) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
6	G	3	BMA	C1-C2	4.61	1.62	1.52
6	G	3	BMA	C2-C3	3.34	1.57	1.52

All (4) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
6	G	3	BMA	C1-C2-C3	3.68	114.19	109.67
6	G	3	BMA	C2-C3-C4	3.61	117.15	110.89
6	G	3	BMA	O2-C2-C1	2.93	115.15	109.15
6	G	3	BMA	C1-O5-C5	-2.38	108.97	112.19

There are no chirality outliers.

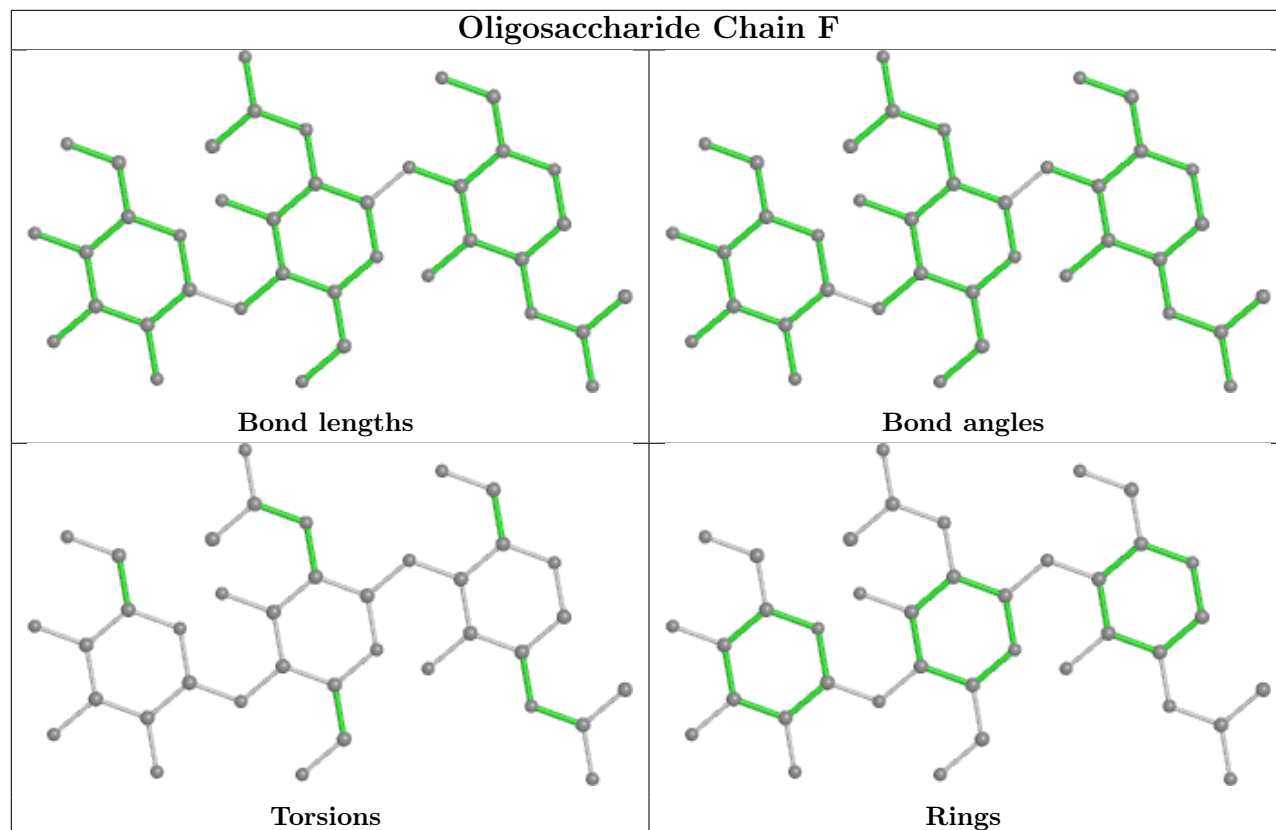
All (4) torsion outliers are listed below:

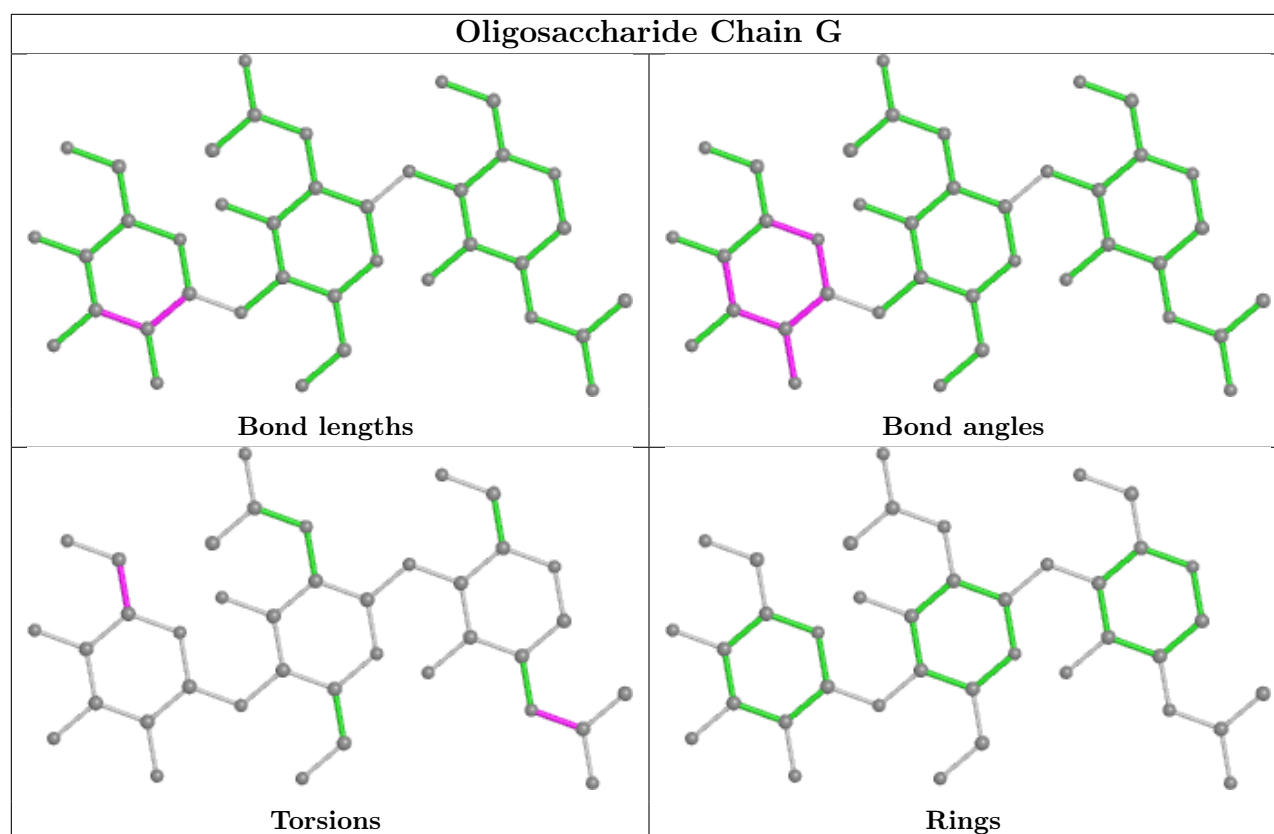
Mol	Chain	Res	Type	Atoms
6	G	3	BMA	C4-C5-C6-O6
6	G	3	BMA	O5-C5-C6-O6
6	G	1	NAG	C8-C7-N2-C2
6	G	1	NAG	O7-C7-N2-C2

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.





## 5.6 Ligand geometry [i](#)

5 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	$\# Z  > 2$
8	HEM	B	601	2	41,50,50	1.51	3 (7%)	45,82,82	1.47	7 (15%)
9	NAG	B	603	2	14,14,15	0.22	0	17,19,21	0.34	0
10	LBN	B	604	-	47,47,51	0.48	0	53,55,59	0.57	1 (1%)
8	HEM	B	602	2	41,50,50	1.50	3 (7%)	45,82,82	1.35	6 (13%)
7	POV	A	601	-	25,25,51	0.74	1 (4%)	28,28,59	0.94	2 (7%)

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
8	HEM	B	601	2	-	1/12/54/54	-
9	NAG	B	603	2	-	1/6/23/26	0/1/1/1
10	LBN	B	604	-	-	13/51/51/55	-
8	HEM	B	602	2	-	4/12/54/54	-
7	POV	A	601	-	-	10/24/24/55	-

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

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
8	B	601	HEM	C3C-C2C	-4.82	1.33	1.40
8	B	602	HEM	C3C-C2C	-4.67	1.33	1.40
8	B	602	HEM	C3C-CAC	3.64	1.55	1.47
8	B	601	HEM	C3C-CAC	3.51	1.55	1.47
8	B	602	HEM	CAB-C3B	2.99	1.55	1.47

The worst 5 of 16 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
7	A	601	POV	O12-P-O11	-3.40	97.69	106.73
8	B	601	HEM	C1B-NB-C4B	2.93	108.10	105.07
8	B	601	HEM	C4D-ND-C1D	2.83	108.00	105.07
8	B	601	HEM	C3B-C2B-C1B	2.80	108.56	106.49
8	B	602	HEM	C4D-ND-C1D	2.76	107.92	105.07

There are no chirality outliers.

5 of 29 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
7	A	601	POV	C1-O11-P-O12
10	B	604	LBN	C1-O1-P1-O3
10	B	604	LBN	O6-C25-O5-C3
10	B	604	LBN	C26-C25-O5-C3
7	A	601	POV	C311-C310-C39-C38

There are no ring outliers.

3 monomers are involved in 6 short contacts:

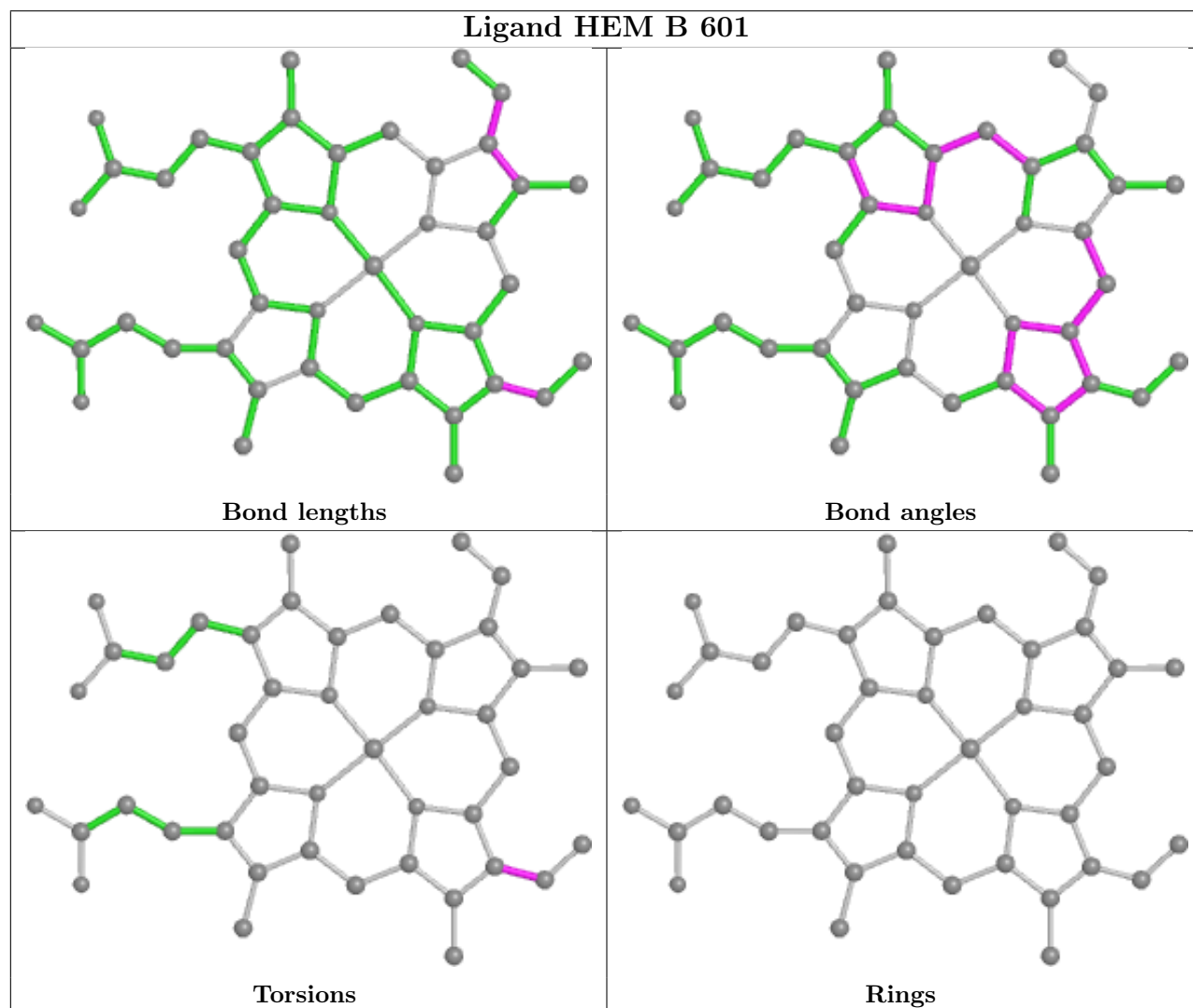
Mol	Chain	Res	Type	Clashes	Symm-Clashes
8	B	601	HEM	3	0
8	B	602	HEM	2	0

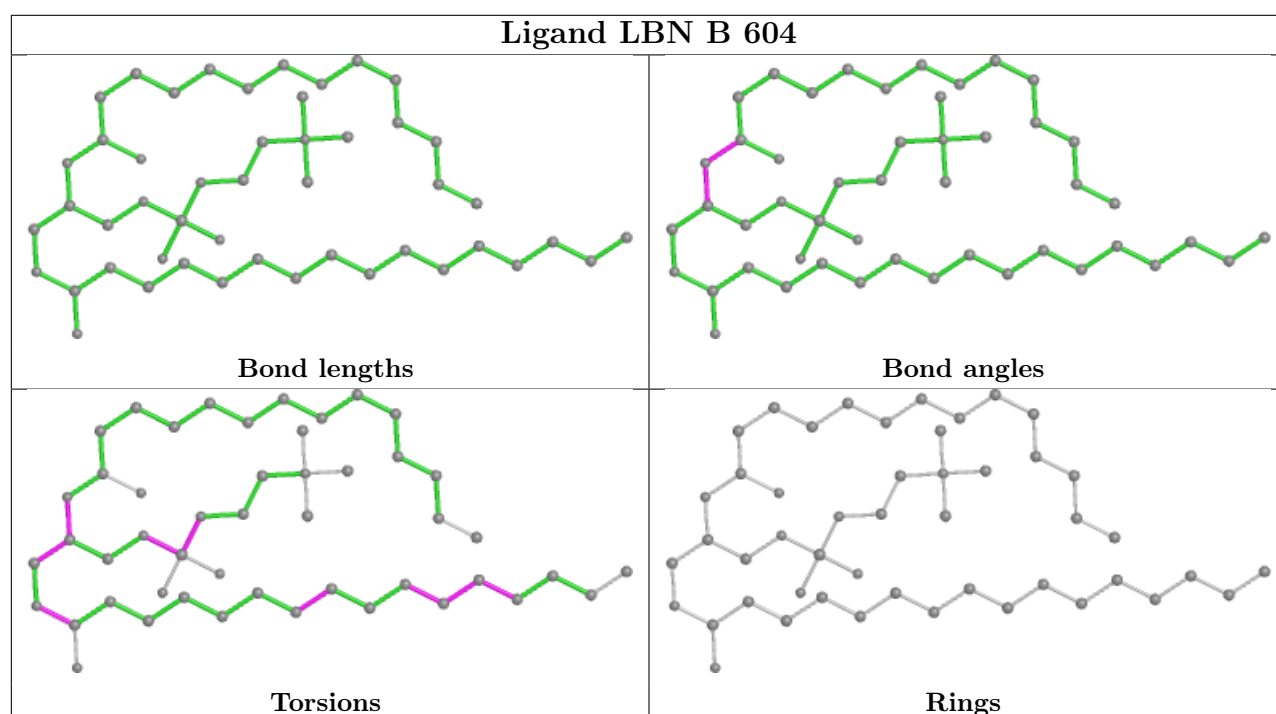
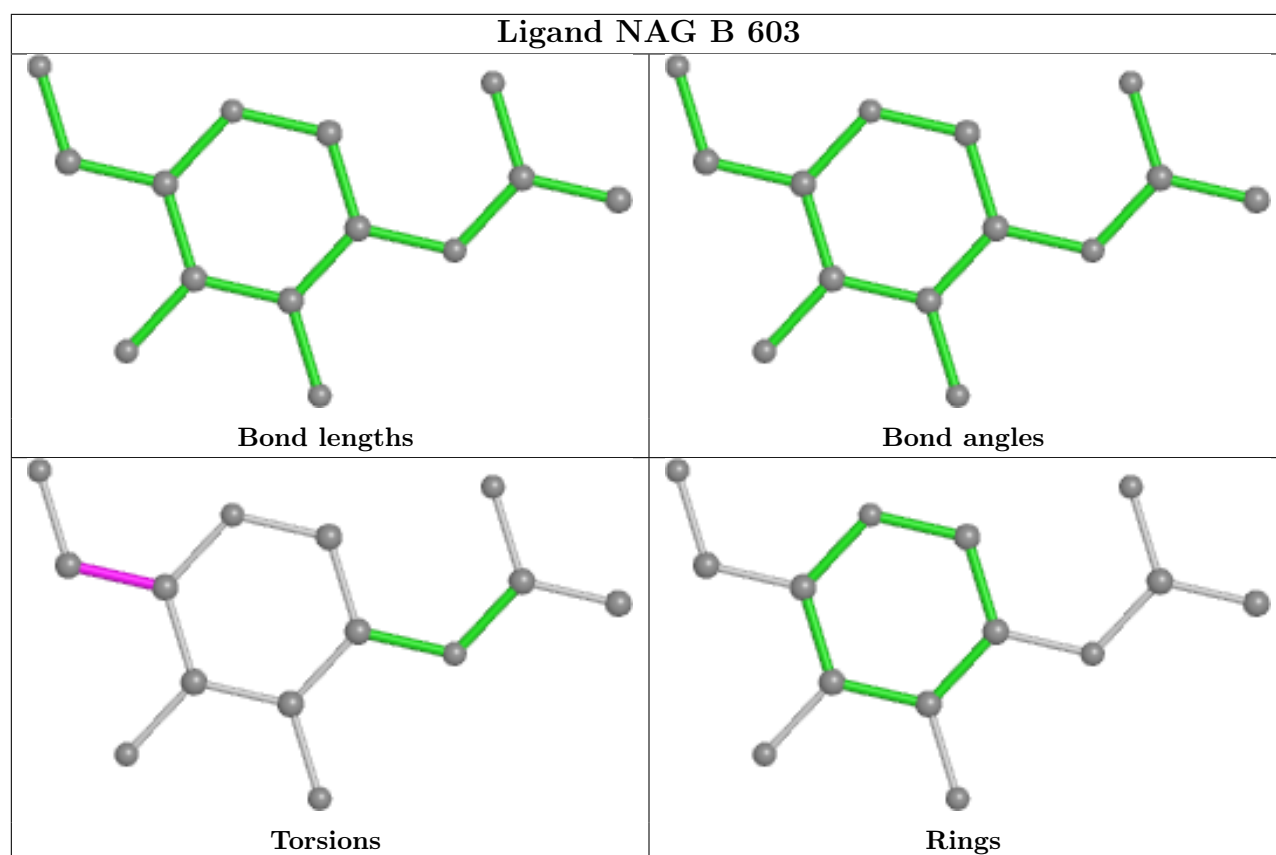
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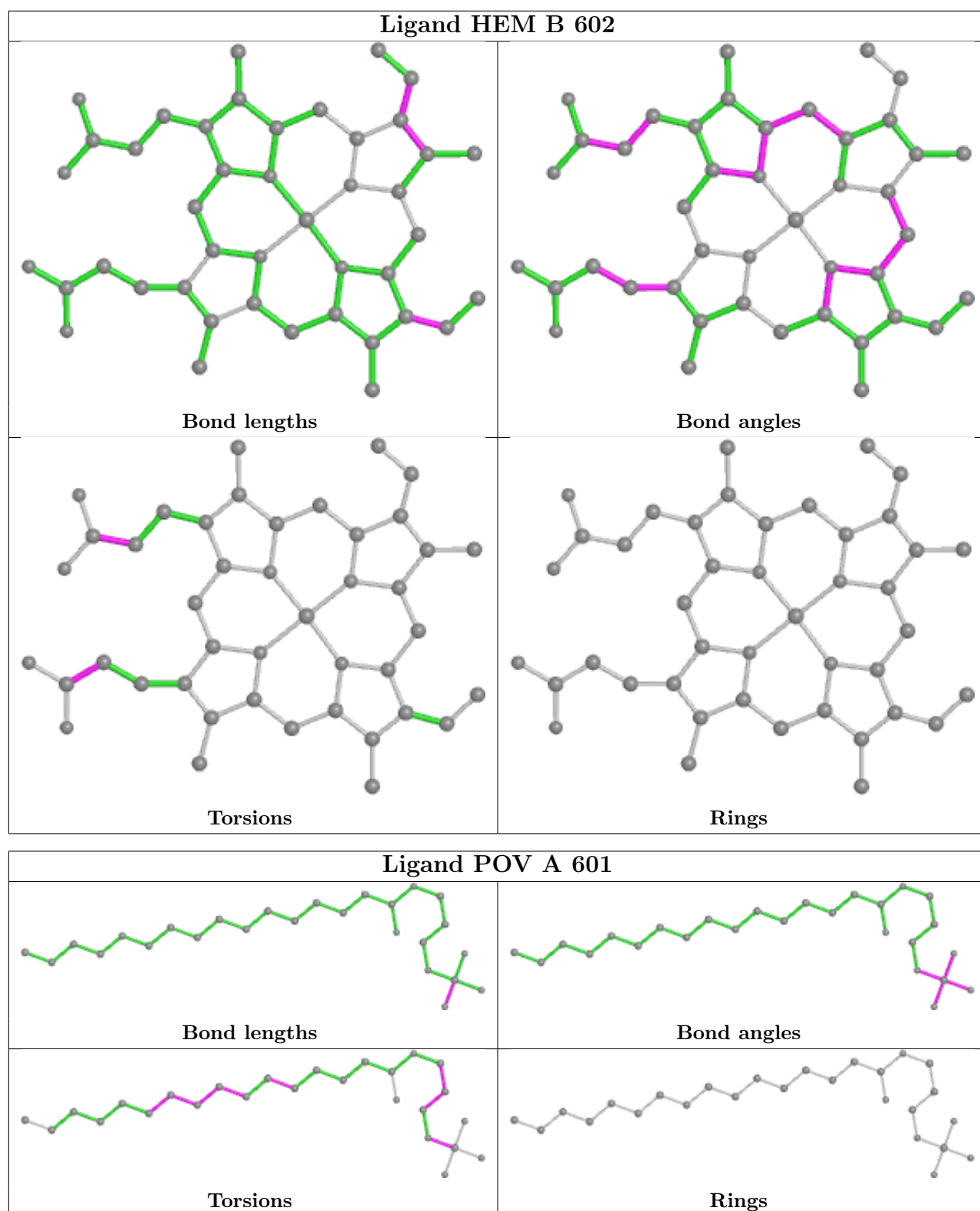
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Mol	Chain	Res	Type	Clashes	Symm-Clashes
7	A	601	POV	1	0

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 than 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.







## 5.7 Other polymers ⓘ

There are no such residues in this entry.

## 5.8 Polymer linkage issues ⓘ

There are no chain breaks in this entry.