wwPDB EM Validation Summary Report

Oct 7, 2024 – 02:37 PM JST

PDB ID	:	8IZD
EMDB ID	:	EMD-35862
Title	:	Cryo-EM structure of the C26-CoA-bound Lac1-Lip1 complex
Authors	:	Xie, T.; Fang, Q.; Gong, X.
Deposited on	:	2023-04-07
Resolution	:	3.09 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*
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.

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

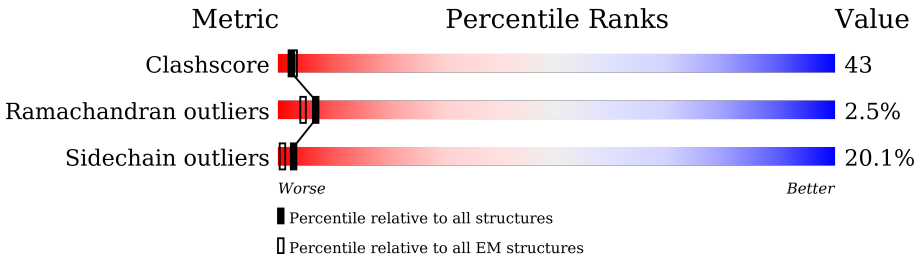
EMDB validation analysis	:	FAILED
Mogul	:	1.8.5 (274361), CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20231227.v01 (using entries in the PDB archive December 27th 2023)
MapQ	:	FAILED
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.39

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 3.09 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 >=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	428	20% 25% 22% 7% 26%
1	C	428	21% 24% 23% 6% 26%
2	B	150	55% 23% 8% • 12%
2	D	150	54% 25% 7% • 12%

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	9NY	A	502	-	-	X	-
4	9NY	C	502	-	-	X	-

2 Entry composition (i)

There are 4 unique types of molecules in this entry. The entry contains 7806 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 Ceramide synthase LAC1.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	315	Total	C	N	O	S	0	0
			2670	1809	424	424	13		
1	C	315	Total	C	N	O	S	0	0
			2670	1809	424	424	13		

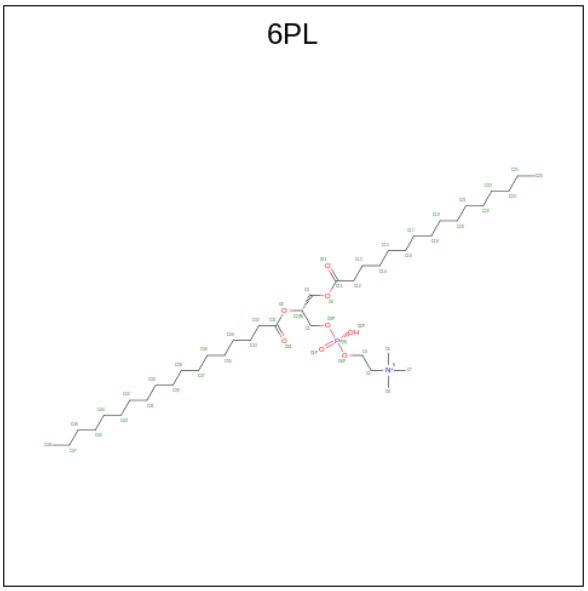
There are 20 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	419	LEU	-	expression tag	UNP P28496
A	420	GLU	-	expression tag	UNP P28496
A	421	ASP	-	expression tag	UNP P28496
A	422	TYR	-	expression tag	UNP P28496
A	423	LYS	-	expression tag	UNP P28496
A	424	ASP	-	expression tag	UNP P28496
A	425	ASP	-	expression tag	UNP P28496
A	426	ASP	-	expression tag	UNP P28496
A	427	ASP	-	expression tag	UNP P28496
A	428	LYS	-	expression tag	UNP P28496
C	419	LEU	-	expression tag	UNP P28496
C	420	GLU	-	expression tag	UNP P28496
C	421	ASP	-	expression tag	UNP P28496
C	422	TYR	-	expression tag	UNP P28496
C	423	LYS	-	expression tag	UNP P28496
C	424	ASP	-	expression tag	UNP P28496
C	425	ASP	-	expression tag	UNP P28496
C	426	ASP	-	expression tag	UNP P28496
C	427	ASP	-	expression tag	UNP P28496
C	428	LYS	-	expression tag	UNP P28496

- Molecule 2 is a protein called Ceramide synthase subunit LIP1.

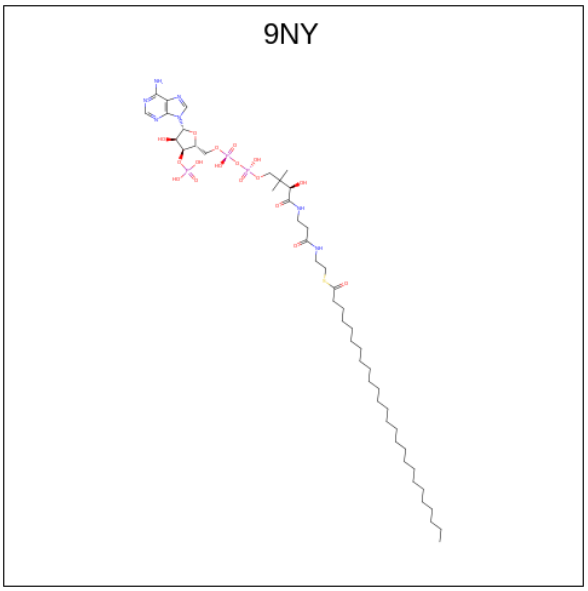
Mol	Chain	Residues	Atoms					AltConf	Trace
2	B	132	Total	C	N	O	S	0	0
			1082	698	177	201	6		
2	D	132	Total	C	N	O	S	0	0
			1082	698	177	201	6		

- Molecule 3 is (4S,7R)-4-HYDROXY-N,N,N-TRIMETHYL-9-OXO-7-[(PALMITOYLOXY) METHYL]-3,5,8-TRIOXA-4-PHOSPHAHEXACOSAN-1-AMINIUM 4-OXIDE (three-letter code: 6PL) (formula: $C_{42}H_{85}NO_8P$).



Mol	Chain	Residues	Atoms				AltConf
3	A	1	Total	C			0
			18	18			
3	B	1	Total	C	O	P	0
			40	31	8	1	
3	B	1	Total	C			0
			18	18			
3	C	1	Total	C			0
			18	18			
3	D	1	Total	C	O	P	0
			40	31	8	1	
3	D	1	Total	C			0
			18	18			

- Molecule 4 is Hexacosanoyl-CoA (three-letter code: 9NY) (formula: $C_{47}H_{86}N_7O_{17}P_3S$) (labeled as "Ligand of Interest" by depositor).



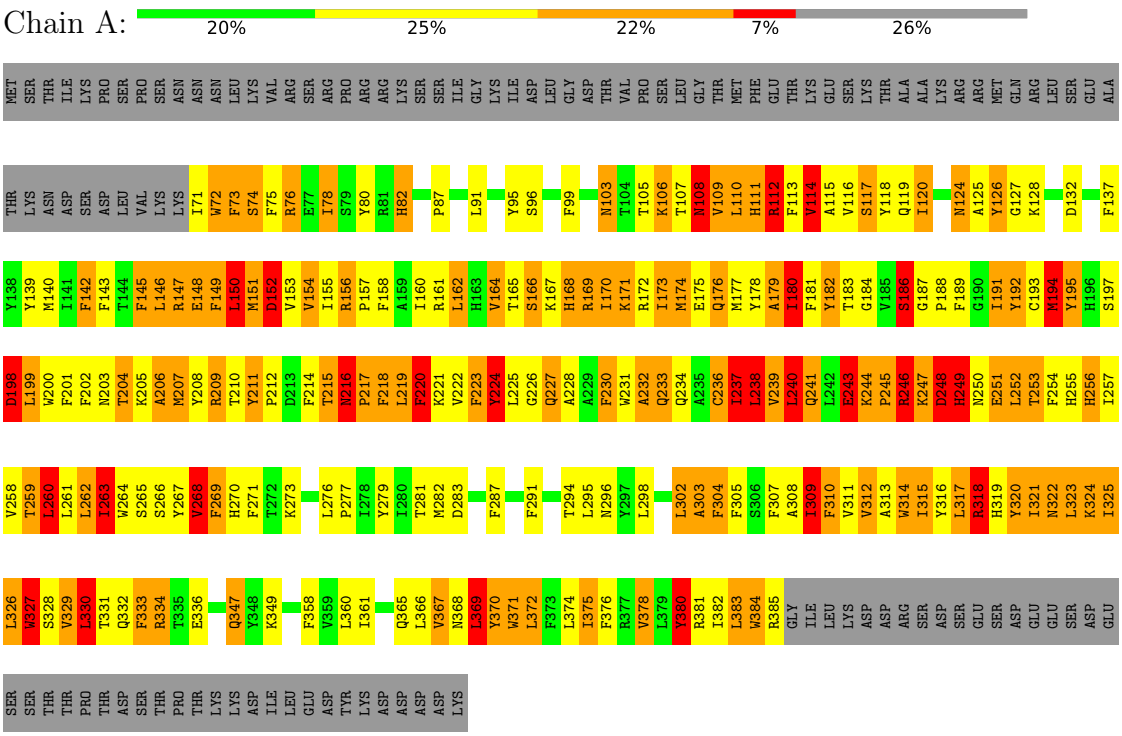
Mol	Chain	Residues	Atoms						AltConf
4	A	1	Total	C	N	O	P	S	0
			75	47	7	17	3	1	
4	C	1	Total	C	N	O	P	S	0
			75	47	7	17	3	1	

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.

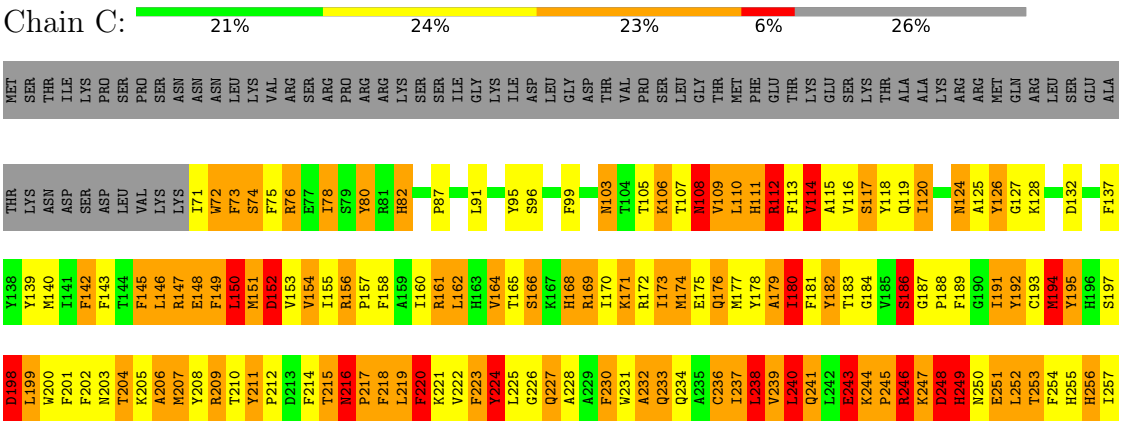
- Molecule 1: Ceramide synthase LAC1

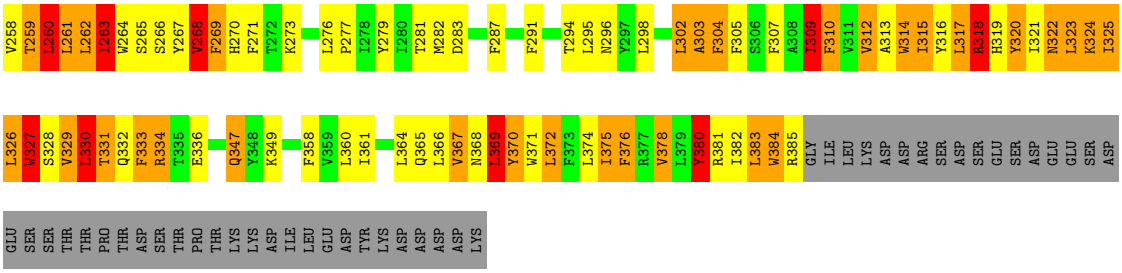
Chain A:



- Molecule 1: Ceramide synthase LAC1

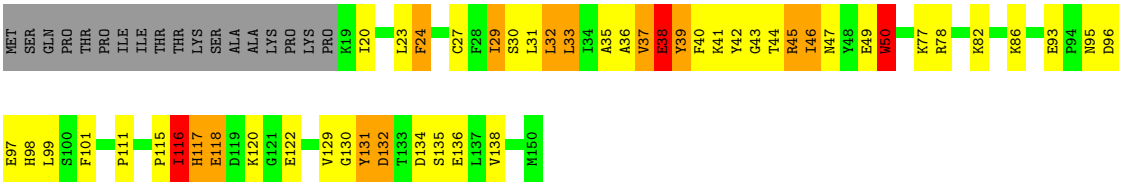
Chain C:





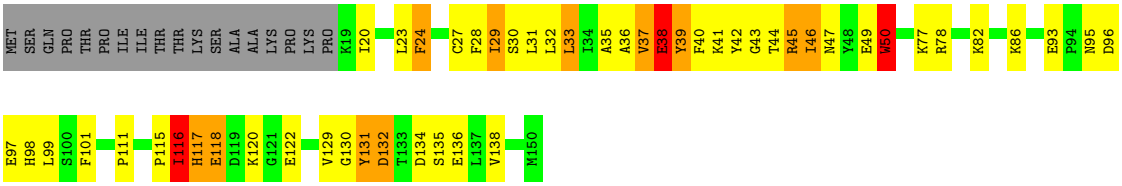
• Molecule 2: Ceramide synthase subunit LIP1

Chain B:



• Molecule 2: Ceramide synthase subunit LIP1

Chain D:



4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	179070	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	NONE	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	50	Depositor
Minimum defocus (nm)	1000	Depositor
Maximum defocus (nm)	2000	Depositor
Magnification	Not provided	
Image detector	GATAN K3 (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: 9NY, 6PL

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	2.90	282/2764 (10.2%)	1.67	72/3760 (1.9%)
1	C	2.90	278/2764 (10.1%)	1.67	72/3760 (1.9%)
2	B	1.91	41/1114 (3.7%)	1.12	8/1512 (0.5%)
2	D	1.91	42/1114 (3.8%)	1.12	9/1512 (0.6%)
All	All	2.66	643/7756 (8.3%)	1.53	161/10544 (1.5%)

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	3
1	C	0	4
All	All	0	7

The worst 5 of 643 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	C	327	TRP	CB-CG	-13.84	1.25	1.50
1	A	327	TRP	CB-CG	-13.79	1.25	1.50
1	C	139	TYR	CE1-CZ	-13.34	1.21	1.38
1	A	139	TYR	CE1-CZ	-13.28	1.21	1.38
1	A	127	GLY	C-O	-12.84	1.03	1.23

The worst 5 of 161 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	C	318	ARG	NE-CZ-NH2	10.33	125.46	120.30
1	A	318	ARG	NE-CZ-NH2	10.25	125.43	120.30

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Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	C	173	ILE	CB-CA-C	-10.23	91.14	111.60
1	A	173	ILE	CB-CA-C	-10.21	91.18	111.60
1	A	317	LEU	CB-CG-CD2	-10.06	93.89	111.00

There are no chirality outliers.

5 of 7 planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	239	VAL	Mainchain
1	A	302	LEU	Mainchain
1	A	318	ARG	Mainchain
1	C	239	VAL	Mainchain
1	C	302	LEU	Mainchain

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	2670	0	2667	284	0
1	C	2670	0	2667	288	0
2	B	1082	0	1037	44	0
2	D	1082	0	1037	45	0
3	A	18	0	35	2	0
3	B	58	0	91	18	0
3	C	18	0	35	1	0
3	D	58	0	91	18	0
4	A	75	0	0	21	0
4	C	75	0	0	21	0
All	All	7806	0	7660	668	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 43.

The worst 5 of 668 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:323:LEU:CD2	1:C:365:GLN:HE21	1.43	1.31
1:A:323:LEU:CD2	1:A:365:GLN:HE21	1.43	1.29
1:C:323:LEU:HD21	1:C:365:GLN:NE2	1.48	1.26
1:A:251:GLU:HG2	1:A:370:TYR:CE2	1.69	1.26
1:A:323:LEU:HD21	1:A:365:GLN:NE2	1.48	1.26

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	313/428 (73%)	266 (85%)	37 (12%)	10 (3%)	3 18
1	C	313/428 (73%)	267 (85%)	36 (12%)	10 (3%)	3 18
2	B	130/150 (87%)	120 (92%)	9 (7%)	1 (1%)	16 48
2	D	130/150 (87%)	120 (92%)	9 (7%)	1 (1%)	16 48
All	All	886/1156 (77%)	773 (87%)	91 (10%)	22 (2%)	7 22

5 of 22 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	245	PRO
1	A	248	ASP
1	A	249	HIS
2	B	120	LYS
1	C	245	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 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	284/391 (73%)	214 (75%)	70 (25%)	0 2
1	C	284/391 (73%)	215 (76%)	69 (24%)	0 2
2	B	119/135 (88%)	108 (91%)	11 (9%)	7 28
2	D	119/135 (88%)	107 (90%)	12 (10%)	6 23
All	All	806/1052 (77%)	644 (80%)	162 (20%)	3 4

5 of 162 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	C	209	ARG
1	C	347	GLN
1	C	224	TYR
1	C	251	GLU
2	D	24	PHE

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 13 such sidechains are listed below:

Mol	Chain	Res	Type
1	C	111	HIS
1	C	131	ASN
2	D	117	HIS
1	C	319	HIS
1	C	365	GLN

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)

There are no oligosaccharides in this entry.

5.6 Ligand geometry (i)

8 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	9NY	C	502	-	69,77,77	2.34	17 (24%)	80,103,103	2.28	24 (30%)
3	6PL	D	202	-	17,17,51	0.29	0	16,16,59	0.85	0
3	6PL	A	501	-	17,17,51	0.22	0	16,16,59	0.63	0
4	9NY	A	502	-	69,77,77	2.34	17 (24%)	80,103,103	2.29	24 (30%)
3	6PL	B	202	-	17,17,51	0.29	0	16,16,59	0.85	0
3	6PL	D	201	-	39,39,51	1.53	6 (15%)	43,44,59	1.86	8 (18%)
3	6PL	C	501	-	17,17,51	0.22	0	16,16,59	0.63	0
3	6PL	B	201	-	39,39,51	1.53	6 (15%)	43,44,59	1.87	8 (18%)

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	9NY	C	502	-	-	35/72/92/92	0/3/3/3
3	6PL	D	202	-	-	6/15/15/55	-
3	6PL	A	501	-	-	8/15/15/55	-
4	9NY	A	502	-	-	35/72/92/92	0/3/3/3
3	6PL	B	202	-	-	6/15/15/55	-
3	6PL	D	201	-	-	21/41/41/55	-
3	6PL	C	501	-	-	8/15/15/55	-
3	6PL	B	201	-	-	21/41/41/55	-

The worst 5 of 46 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
4	A	502	9NY	C44-S1	9.36	1.98	1.76

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Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
4	C	502	9NY	C44-S1	9.35	1.98	1.76
4	C	502	9NY	C40-C44	7.41	1.58	1.50
4	A	502	9NY	C40-C44	7.39	1.58	1.50
4	C	502	9NY	O12-C25	-5.72	1.31	1.42

The worst 5 of 64 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
4	C	502	9NY	C5-N5-C1	6.27	137.66	126.64
4	A	502	9NY	C5-N5-C1	6.27	137.65	126.64
4	A	502	9NY	O11-C24-C11	6.16	120.45	110.55
4	C	502	9NY	O11-C24-C11	6.13	120.40	110.55
4	A	502	9NY	N2-C6-N1	-6.10	119.15	128.68

There are no chirality outliers.

5 of 140 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	B	201	6PL	O31-C31-O2-C2
3	B	201	6PL	C32-C31-O2-C2
3	D	201	6PL	O31-C31-O2-C2
3	D	201	6PL	C32-C31-O2-C2
4	A	502	9NY	C24-C11-C25-C36

There are no ring outliers.

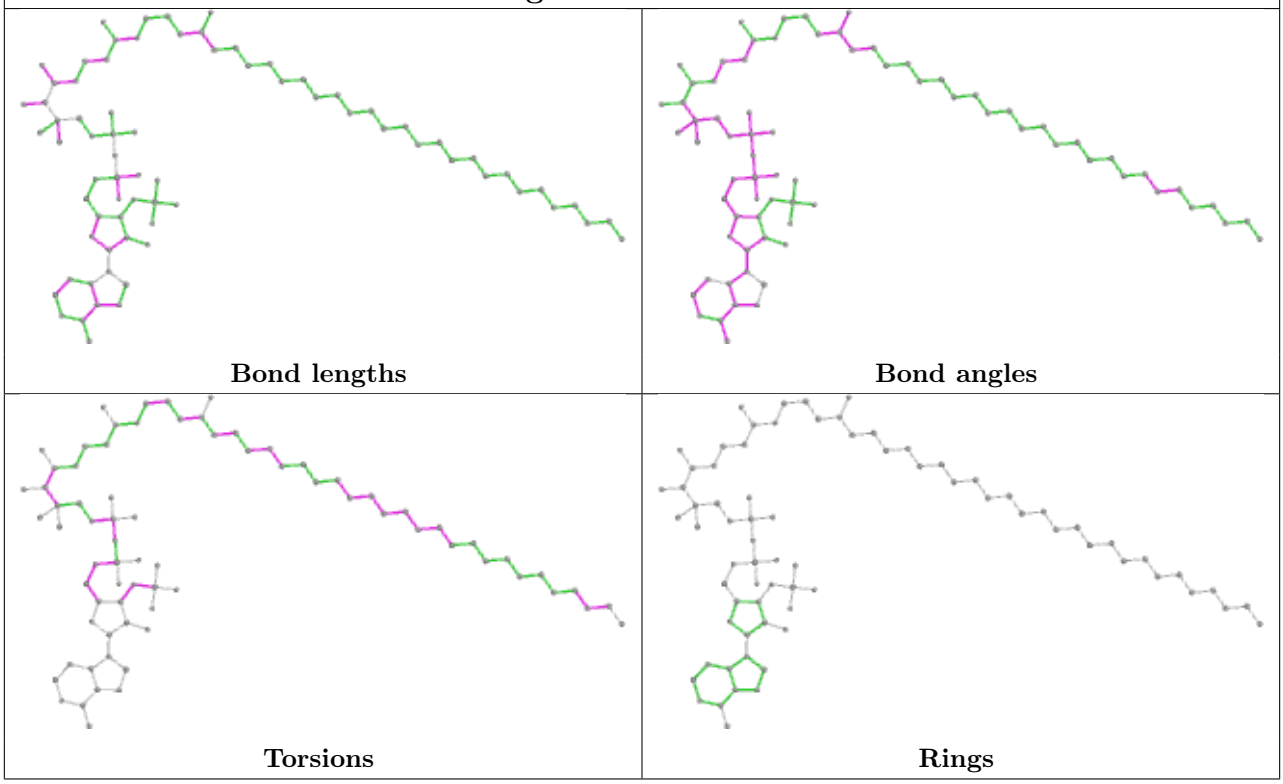
8 monomers are involved in 81 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	C	502	9NY	21	0
3	D	202	6PL	2	0
3	A	501	6PL	2	0
4	A	502	9NY	21	0
3	B	202	6PL	2	0
3	D	201	6PL	16	0
3	C	501	6PL	1	0
3	B	201	6PL	16	0

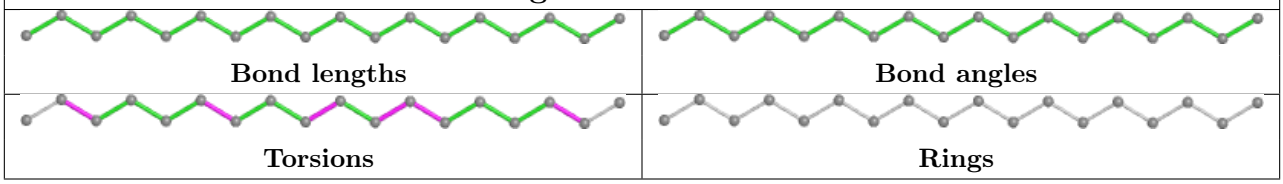
The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. 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.

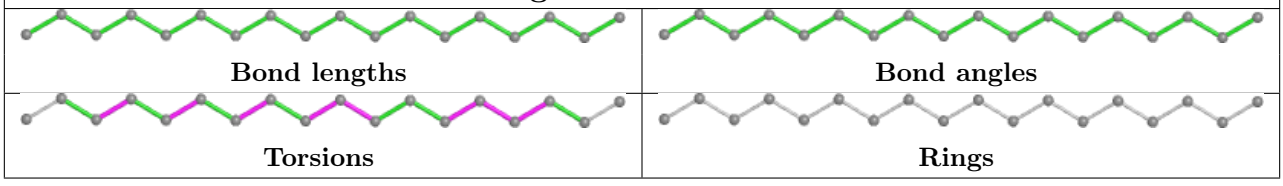
Ligand 9NY C 502



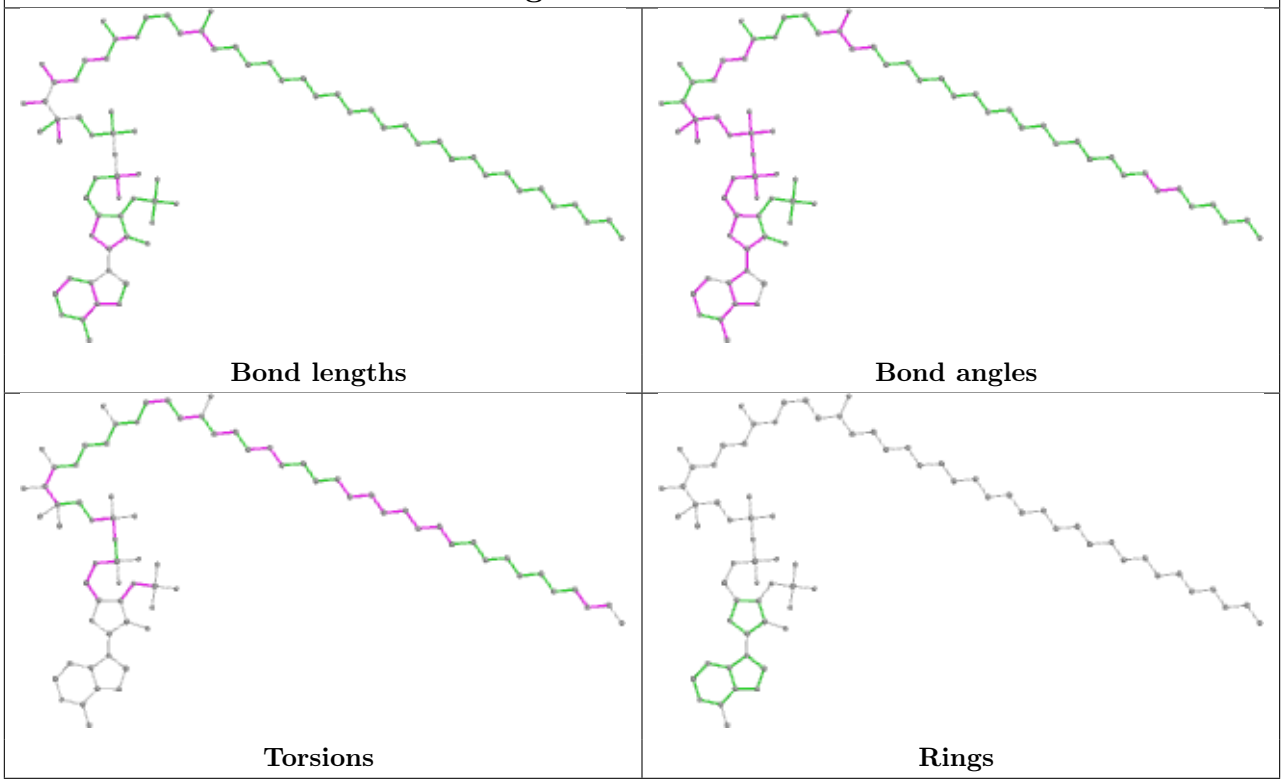
Ligand 6PL D 202



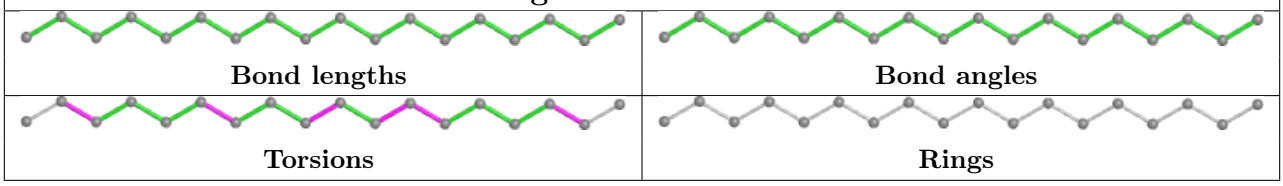
Ligand 6PL A 501



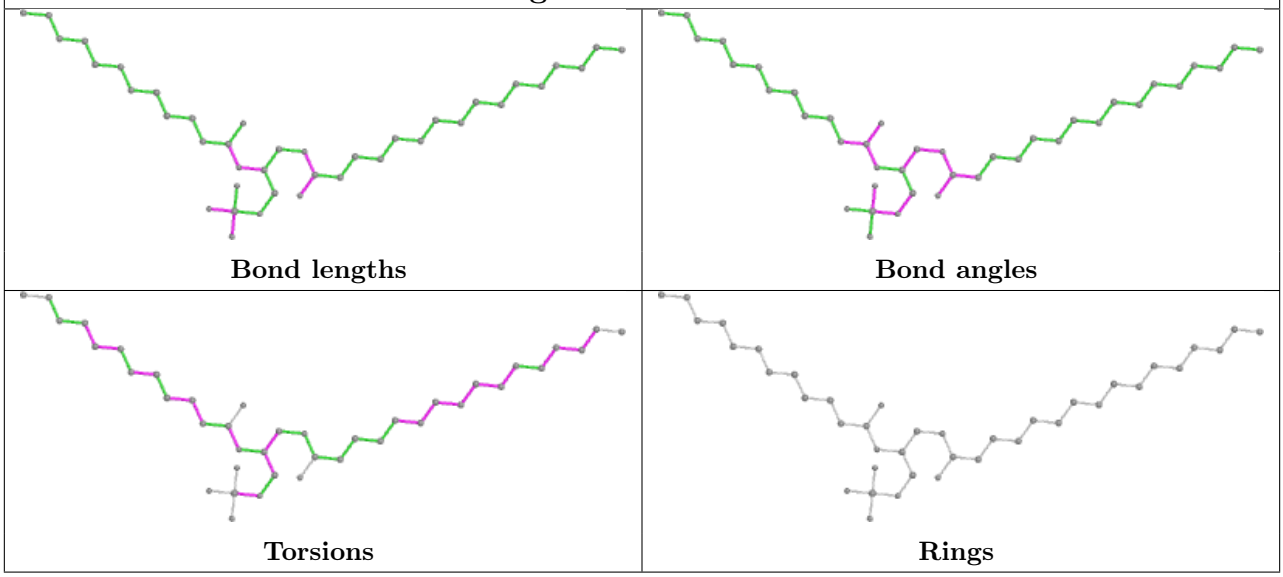
Ligand 9NY A 502

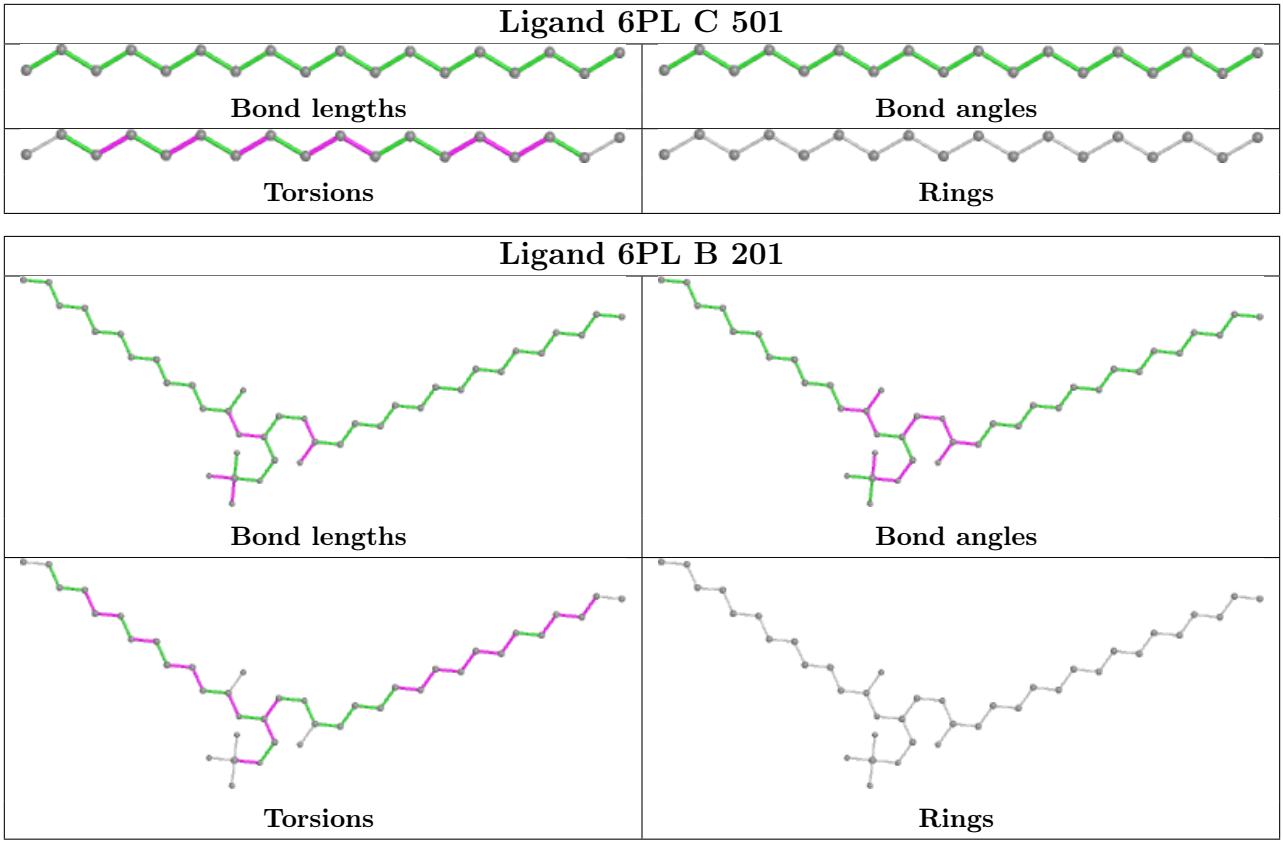


Ligand 6PL B 202



Ligand 6PL D 201





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.