



wwPDB EM Validation Summary Report ⓘ

Nov 13, 2024 – 10:25 AM JST

PDB ID : 8YUT
EMDB ID : EMD-39582
Title : Cryo-EM structure of the amthamine-bound H2R-Gs complex
Authors : Shen, Q.; Tang, X.; Wen, X.; Cheng, S.; Xiao, P.; Zang, S.; Shen, D.; Jiang, L.; Zheng, Y.; Zhang, H.; Xu, H.; Mao, C.; Zhang, M.; Hu, W.; Sun, J.; Chen, Z.; Zhang, Y.
Deposited on : 2024-03-27
Resolution : 2.70 Å(reported)

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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

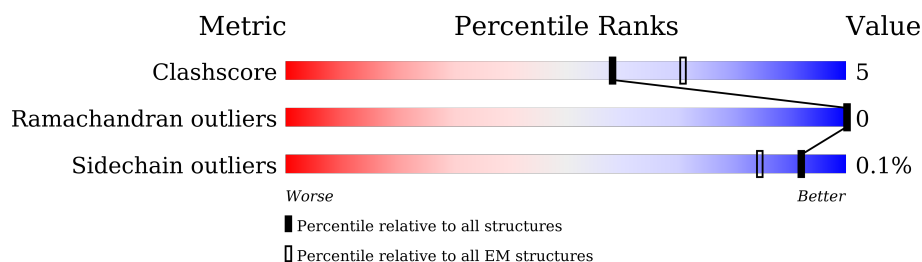
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

ELECTRON MICROSCOPY

The reported resolution of this entry is 2.70 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 $\leq 5\%$

Mol	Chain	Length	Quality of chain
1	A	378	
2	B	356	
3	G	71	
4	N	128	
5	R	411	

2 Entry composition

There are 7 unique types of molecules in this entry. The entry contains 8229 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 Guanine nucleotide-binding protein G(s) subunit alpha isoforms short.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	227	Total	C	N	O	S	0	0
			1891	1193	344	347	7		

- Molecule 2 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	B	338	Total	C	N	O	S	0	0
			2601	1604	467	509	21		

There are 17 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	-15	MET	-	initiating methionine	UNP P62873
B	-14	HIS	-	expression tag	UNP P62873
B	-13	HIS	-	expression tag	UNP P62873
B	-12	HIS	-	expression tag	UNP P62873
B	-11	HIS	-	expression tag	UNP P62873
B	-10	LEU	-	expression tag	UNP P62873
B	-9	GLU	-	expression tag	UNP P62873
B	-8	VAL	-	expression tag	UNP P62873
B	-7	LEU	-	expression tag	UNP P62873
B	-6	PHE	-	expression tag	UNP P62873
B	-5	GLN	-	expression tag	UNP P62873
B	-4	GLY	-	expression tag	UNP P62873
B	-3	PRO	-	expression tag	UNP P62873
B	-2	GLY	-	expression tag	UNP P62873
B	-1	SER	-	expression tag	UNP P62873
B	0	SER	-	expression tag	UNP P62873
B	1	GLY	-	expression tag	UNP P62873

- Molecule 3 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2.

Mol	Chain	Residues	Atoms					AltConf	Trace
3	G	57	Total	C	N	O	S	0	0
			436	273	77	83	3		

- Molecule 4 is a protein called Nanobody-35.

Mol	Chain	Residues	Atoms					AltConf	Trace
4	N	126	Total	C	N	O	S	0	0
			961	599	168	188	6		

- Molecule 5 is a protein called Histamine H2 receptor.

Mol	Chain	Residues	Atoms					AltConf	Trace
5	R	293	Total	C	N	O	S	0	0
			2302	1510	381	394	17		

There are 53 discrepancies between the modelled and reference sequences:

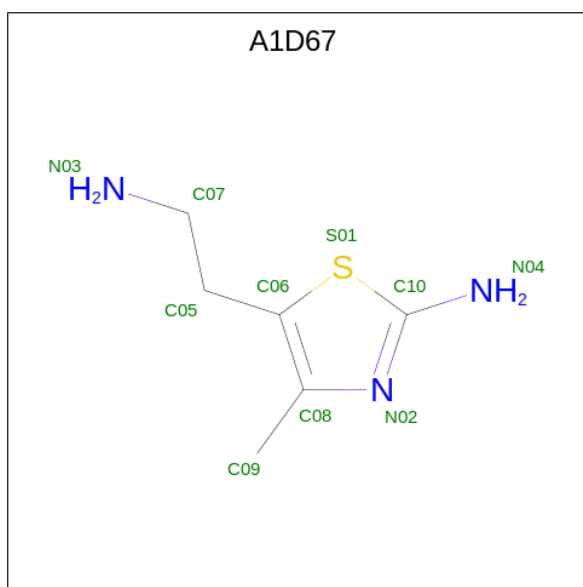
Chain	Residue	Modelled	Actual	Comment	Reference
R	-32	MET	-	initiating methionine	UNP P25021
R	-31	LYS	-	expression tag	UNP P25021
R	-30	THR	-	expression tag	UNP P25021
R	-29	ILE	-	expression tag	UNP P25021
R	-28	ILE	-	expression tag	UNP P25021
R	-27	ALA	-	expression tag	UNP P25021
R	-26	LEU	-	expression tag	UNP P25021
R	-25	SER	-	expression tag	UNP P25021
R	-24	TYR	-	expression tag	UNP P25021
R	-23	ILE	-	expression tag	UNP P25021
R	-22	PHE	-	expression tag	UNP P25021
R	-21	CYS	-	expression tag	UNP P25021
R	-20	LEU	-	expression tag	UNP P25021
R	-19	VAL	-	expression tag	UNP P25021
R	-18	PHE	-	expression tag	UNP P25021
R	-17	ALA	-	expression tag	UNP P25021
R	-16	ASP	-	expression tag	UNP P25021
R	-15	TYR	-	expression tag	UNP P25021
R	-14	LYS	-	expression tag	UNP P25021
R	-13	ASP	-	expression tag	UNP P25021
R	-12	ASP	-	expression tag	UNP P25021
R	-11	ASP	-	expression tag	UNP P25021
R	-10	ASP	-	expression tag	UNP P25021
R	-9	LYS	-	expression tag	UNP P25021
R	-8	GLU	-	expression tag	UNP P25021

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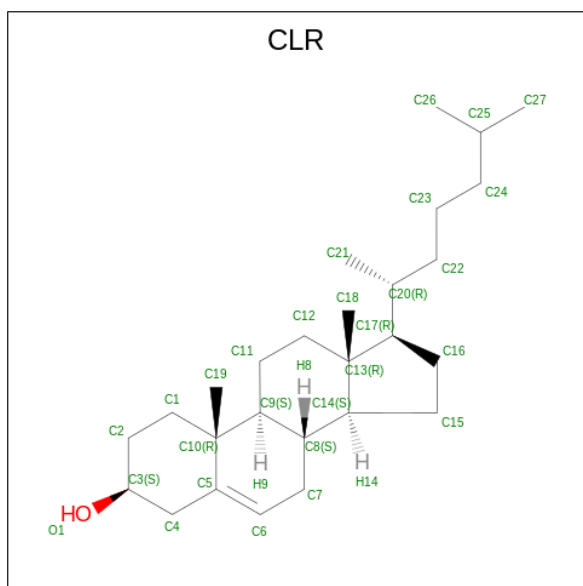
Chain	Residue	Modelled	Actual	Comment	Reference
R	-7	PHE	-	expression tag	UNP P25021
R	-6	LEU	-	expression tag	UNP P25021
R	-5	GLU	-	expression tag	UNP P25021
R	-4	VAL	-	expression tag	UNP P25021
R	-3	LEU	-	expression tag	UNP P25021
R	-2	PHE	-	expression tag	UNP P25021
R	-1	GLN	-	expression tag	UNP P25021
R	0	GLY	-	expression tag	UNP P25021
R	1	PRO	-	expression tag	UNP P25021
R	360	LEU	-	expression tag	UNP P25021
R	361	GLU	-	expression tag	UNP P25021
R	362	GLU	-	expression tag	UNP P25021
R	363	ASN	-	expression tag	UNP P25021
R	364	LEU	-	expression tag	UNP P25021
R	365	TYR	-	expression tag	UNP P25021
R	366	PHE	-	expression tag	UNP P25021
R	367	GLN	-	expression tag	UNP P25021
R	368	GLY	-	expression tag	UNP P25021
R	369	HIS	-	expression tag	UNP P25021
R	370	HIS	-	expression tag	UNP P25021
R	371	HIS	-	expression tag	UNP P25021
R	372	HIS	-	expression tag	UNP P25021
R	373	HIS	-	expression tag	UNP P25021
R	374	HIS	-	expression tag	UNP P25021
R	375	HIS	-	expression tag	UNP P25021
R	376	HIS	-	expression tag	UNP P25021
R	377	HIS	-	expression tag	UNP P25021
R	378	HIS	-	expression tag	UNP P25021

- Molecule 6 is 5-(2-azanylethyl)-4-methyl-1,3-thiazol-2-amine (three-letter code: A1D67) (formula: C₆H₁₁N₃S) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms				AltConf
6	R	1	Total	C	N	S	0
			10	6	3	1	

- Molecule 7 is CHOLESTEROL (three-letter code: CLR) (formula: $C_{27}H_{46}O$).

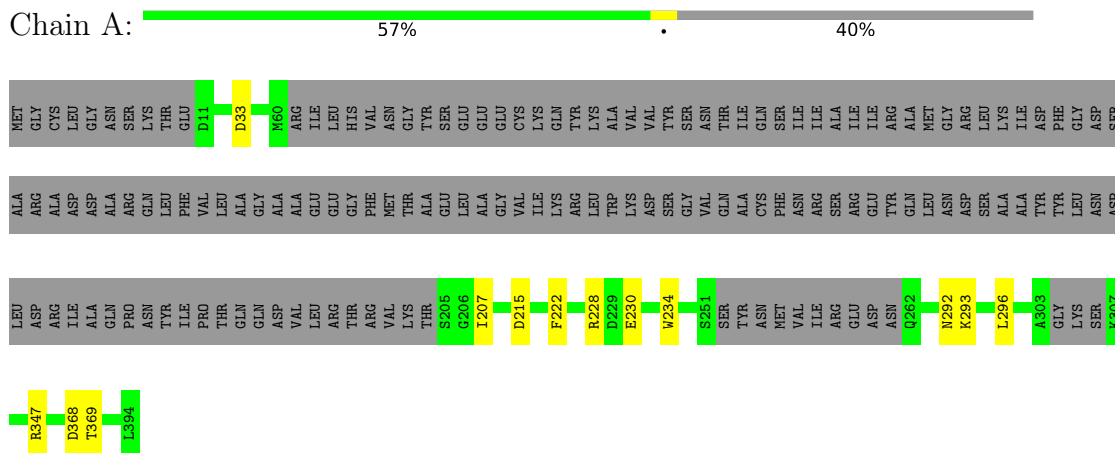


Mol	Chain	Residues	Atoms				AltConf
7	R	1	Total	C	O		0
			28	27	1		

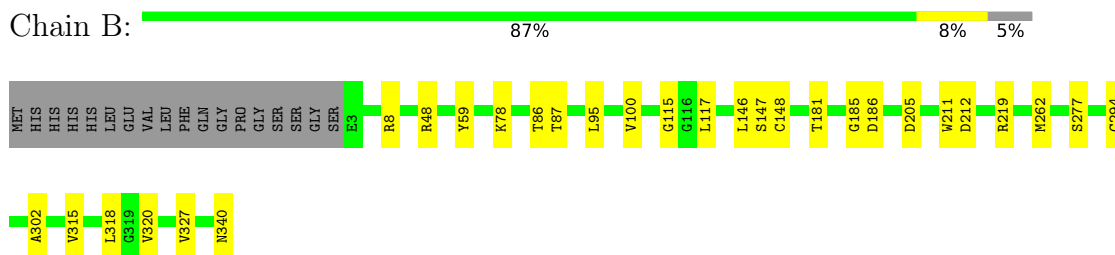
3 Residue-property plots [i](#)

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.

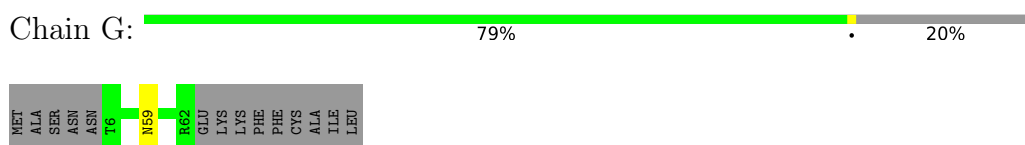
- Molecule 1: Guanine nucleotide-binding protein G(s) subunit alpha isoforms short



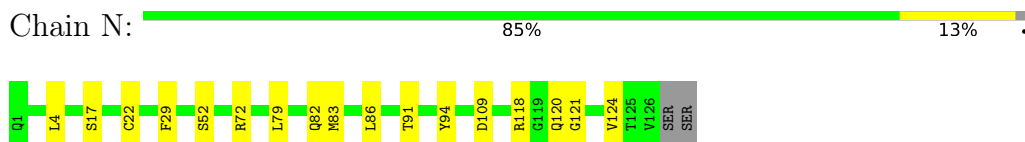
- Molecule 2: Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1



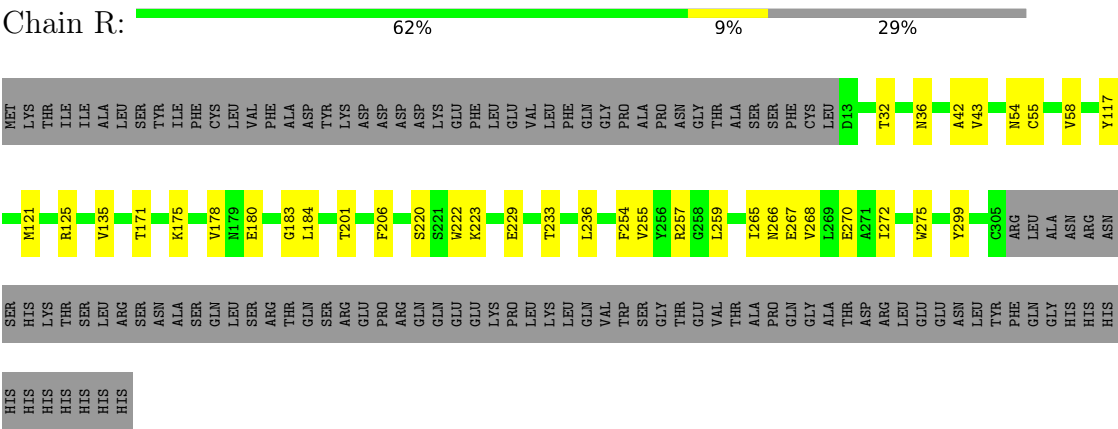
- Molecule 3: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2



- Molecule 4: Nanobody-35



● Molecule 5: Histamine H2 receptor



4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	368447	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 ($e^-/\text{\AA}^2$)	62	Depositor
Minimum defocus (nm)	500	Depositor
Maximum defocus (nm)	2500	Depositor
Magnification	Not provided	
Image detector	GATAN K2 SUMMIT (4k 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: A1D67, CLR

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	0.28	0/1925	0.42	0/2585
2	B	0.31	0/2648	0.52	0/3589
3	G	0.23	0/442	0.37	0/597
4	N	0.27	0/981	0.51	0/1329
5	R	0.38	0/2351	0.48	0/3208
All	All	0.32	0/8347	0.48	0/11308

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	1891	0	1875	11	0
2	B	2601	0	2505	20	0
3	G	436	0	448	1	0
4	N	961	0	928	13	0
5	R	2302	0	2392	38	0
6	R	10	0	0	0	0
7	R	28	0	46	9	0
All	All	8229	0	8194	74	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 5.

The worst 5 of 74 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)
5:R:275:TRP:HB2	7:R:402:CLR:H181	1.50	0.91
5:R:55:CYS:SG	5:R:135:VAL:HG11	2.21	0.80
4:N:17:SER:HA	4:N:83:MET:O	1.83	0.79
5:R:184:LEU:HD21	5:R:259:LEU:HD21	1.69	0.74
5:R:268:VAL:HG23	7:R:402:CLR:H22	1.71	0.73

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	219/378 (58%)	216 (99%)	3 (1%)	0	100	100
2	B	336/356 (94%)	330 (98%)	6 (2%)	0	100	100
3	G	55/71 (78%)	55 (100%)	0	0	100	100
4	N	124/128 (97%)	120 (97%)	4 (3%)	0	100	100
5	R	291/411 (71%)	285 (98%)	6 (2%)	0	100	100
All	All	1025/1344 (76%)	1006 (98%)	19 (2%)	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	205/331 (62%)	205 (100%)	0	100	100
2	B	281/296 (95%)	280 (100%)	1 (0%)	89	96
3	G	46/58 (79%)	46 (100%)	0	100	100
4	N	104/106 (98%)	104 (100%)	0	100	100
5	R	255/361 (71%)	255 (100%)	0	100	100
All	All	891/1152 (77%)	890 (100%)	1 (0%)	92	98

All (1) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	B	59	TYR

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](#)

There are no oligosaccharides in this entry.

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	$\# Z > 2$
7	CLR	R	402	-	31,31,31	3.66	10 (32%)	48,48,48	1.61	11 (22%)
6	A1D67	R	401	-	6,10,10	5.64	3 (50%)	2,13,13	1.95	1 (50%)

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
7	CLR	R	402	-	-	3/10/68/68	0/4/4/4
6	A1D67	R	401	-	-	1/2/3/3	0/1/1/1

The worst 5 of 13 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
6	R	401	A1D67	C06-S01	-12.22	1.51	1.74
7	R	402	CLR	C10-C9	-9.09	1.40	1.56
7	R	402	CLR	C11-C9	8.84	1.68	1.53
7	R	402	CLR	C7-C8	-8.07	1.39	1.53
7	R	402	CLR	C6-C5	6.97	1.48	1.33

The worst 5 of 12 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
7	R	402	CLR	C7-C6-C5	-4.06	117.56	125.06
7	R	402	CLR	C12-C13-C17	-3.70	111.04	116.57
7	R	402	CLR	C15-C14-C8	-3.31	113.63	119.08
7	R	402	CLR	C16-C17-C20	-3.15	107.27	112.15
7	R	402	CLR	C21-C20-C22	-3.02	105.63	110.36

There are no chirality outliers.

All (4) torsion outliers are listed below:

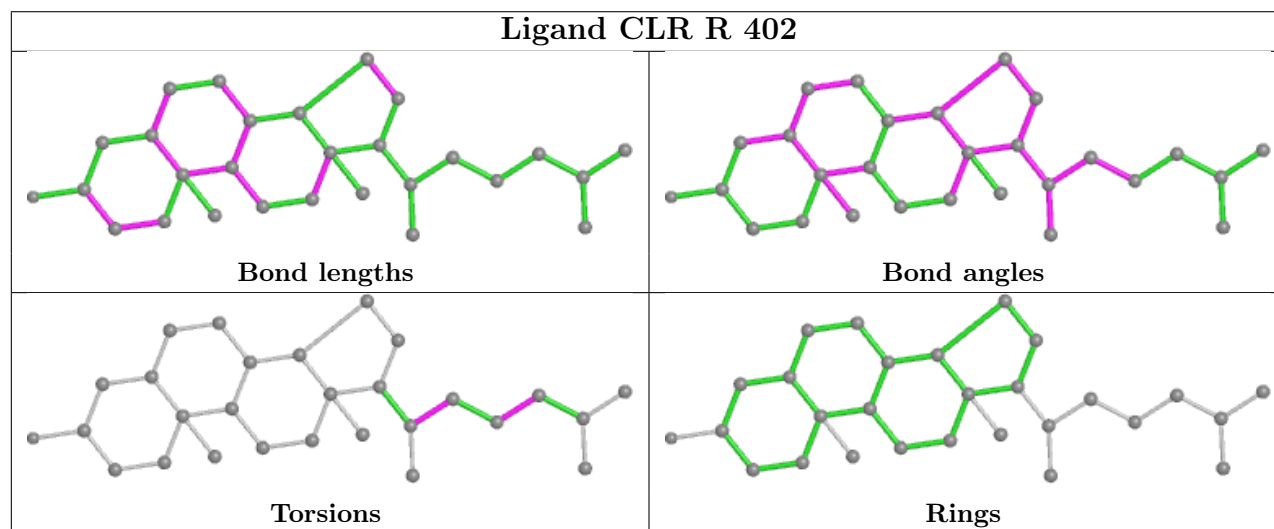
Mol	Chain	Res	Type	Atoms
6	R	401	A1D67	C07-C05-C06-C08
7	R	402	CLR	C17-C20-C22-C23
7	R	402	CLR	C21-C20-C22-C23
7	R	402	CLR	C22-C23-C24-C25

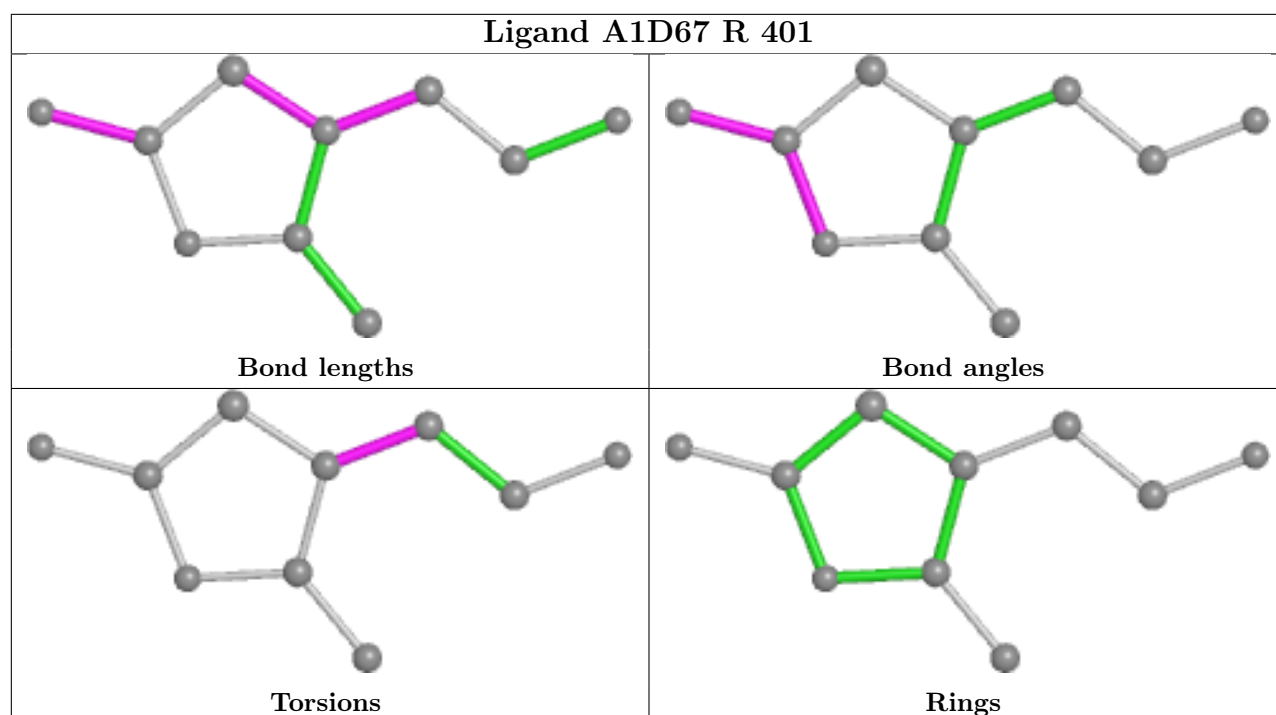
There are no ring outliers.

1 monomer is involved in 9 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
7	R	402	CLR	9	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 [i](#)

There are no such residues in this entry.

5.8 Polymer linkage issues [i](#)

There are no chain breaks in this entry.