

wwPDB EM Validation Summary Report (i)

Oct 28, 2024 – 02:54 PM JST

PDB ID : 9IJD

EMDB ID : EMD-60628

Title : Carazolol-activated human beta3 adrenergic receptor

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Deposited on : 2024-06-22

Resolution : 2.76 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 (i) 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 (1)) 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)

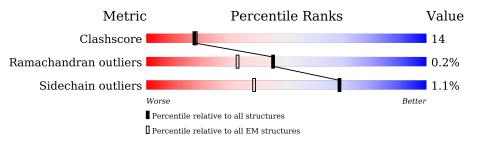
Validation Pipeline (wwPDB-VP) : 2.39

1 Overall quality at a glance (i)

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

The reported resolution of this entry is 2.76 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 $(\# \mathrm{Entries})$	${ m EM\ structures} \ (\#{ m Entries})$
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%





2 Entry composition (i)

There are 7 unique types of molecules in this entry. The entry contains 9371 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(I)/G(S)/G(T) subunit beta-1.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	В	340	Total 2543	C 1576	N 449	O 497	S 21	0	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
В	1	GLN	-	expression tag	UNP P54311

• Molecule 2 is a protein called Camelid antibody VHH fragment.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	N	128	Total 950	C 594	N 166	O 184	S 6	0	0

• Molecule 3 is a protein called Single-chain Fv16.

Mol	Chain	Residues	Atoms					AltConf	Trace
3	D	231	Total 1762	C 1121	N 294	O 337	S 10	0	0

• Molecule 4 is a protein called Beta-3 adrenergic receptor.

Mol	Chain	Residues	Atoms					AltConf	Trace
4	R	252	Total 1893	C 1250	N 320	O 310	S 13	0	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
R	126	TRP	GLU	conflict	UNP P13945

• Molecule 5 is a protein called Guanine nucleotide-binding protein G(s) subunit alpha isoforms



short.

Mol	Chain	Residues	Atoms					AltConf	Trace
5	A	224	Total 1808	C 1146	N 331	O 324	S 7	0	0

There are 30 discrepancies between the modelled and reference sequences:

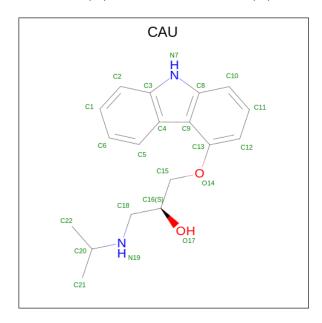
Chain	Residue	Modelled	Actual	Comment	Reference
A	12	LEU	-	expression tag	UNP P04896
A	13	SER	-	expression tag	UNP P04896
A	14	ALA	-	expression tag	UNP P04896
A	15	GLU	-	expression tag	UNP P04896
A	16	ASP	-	expression tag	UNP P04896
A	17	LYS	-	expression tag	UNP P04896
A	18	ALA	-	expression tag	UNP P04896
A	19	ALA	-	expression tag	UNP P04896
A	20	VAL	-	expression tag	UNP P04896
A	21	GLU	-	expression tag	UNP P04896
A	22	ARG	-	expression tag	UNP P04896
A	23	SER	-	expression tag	UNP P04896
A	24	LYS	-	expression tag	UNP P04896
A	25	MET	-	expression tag	UNP P04896
A	49	ASP	GLY	conflict	UNP P04896
A	50	ASN	GLU	conflict	UNP P04896
A	249	ASP	ALA	conflict	UNP P04896
A	252	ASP	SER	conflict	UNP P04896
A	?	-	ASN	deletion	UNP P04896
A	?	-	MET	deletion	UNP P04896
A	?	-	VAL	deletion	UNP P04896
A	?	-	ILE	deletion	UNP P04896
A	?	-	ARG	deletion	UNP P04896
A	?	-	GLU	deletion	UNP P04896
A	?	-	ASP	deletion	UNP P04896
A	?	-	ASN	deletion	UNP P04896
A	?	-	GLN	deletion	UNP P04896
A	?	-	THR	deletion	UNP P04896
A	362	ALA	ILE	conflict	UNP P04896
A	365	ILE	VAL	conflict	UNP P04896

 \bullet Molecule 6 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2.



Mol	Chain	Residues		Atoms					Trace
6	С	52	Total	С	N	О	S	0	0
0	C	92	393	248	66	76	3	U	U

• Molecule 7 is (2S)-1-(9H-Carbazol-4-yloxy)-3-(isopropylamino)propan-2-ol (three-letter code: CAU) (formula: $C_{18}H_{22}N_2O_2$) (labeled as "Ligand of Interest" by depositor).



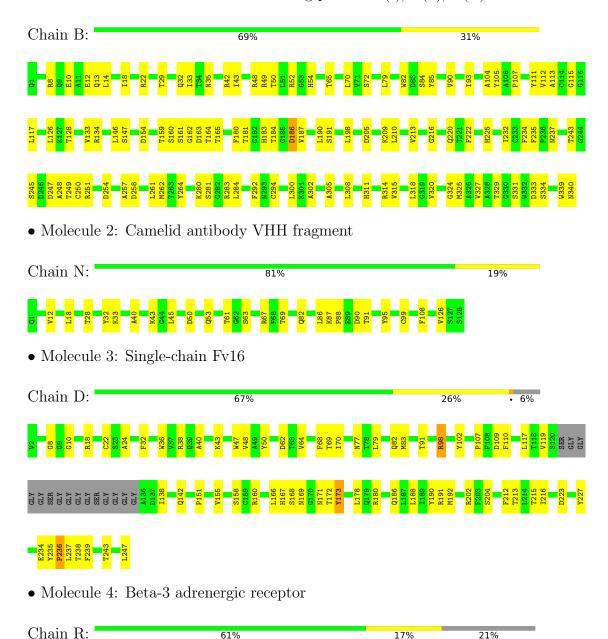
Mol	Chain	Residues	A	AltConf			
7	D	1	Total	С	N	О	0
1	n	1	22	18	2	2	U



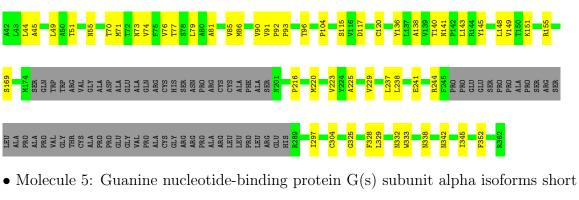
3 Residue-property plots (i)

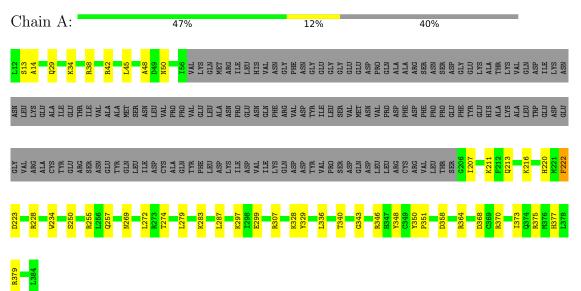
These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry. Residues are color-coded 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(I)/G(S)/G(T) subunit beta-1









• Molecule 6: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2

Chain C: 90% 10%



4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	1623386	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	NONE	Depositor
Microscope	FEI TITAN	Depositor
Voltage (kV)	300	Depositor
Electron dose $(e^-/\text{Å}^2)$	50	Depositor
Minimum defocus (nm)	1100	Depositor
Maximum defocus (nm)	1500	Depositor
Magnification	Not provided	
Image detector	GATAN K3 (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: CAU

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		
IVIOI	Chain	RMSZ	# Z > 5	RMSZ	# Z > 5	
1	В	0.38	0/2590	0.55	0/3521	
2	N	0.36	0/970	0.54	0/1315	
3	D	0.36	0/1806	0.55	0/2450	
4	R	0.38	0/1936	0.57	0/2654	
5	A	0.32	0/1844	0.52	0/2487	
6	С	0.25	0/399	0.39	0/540	
All	All	0.36	0/9545	0.54	0/12967	

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	В	2543	0	2410	86	0
2	N	950	0	897	19	0
3	D	1762	0	1695	74	0
4	R	1893	0	1978	44	0
5	A	1808	0	1770	36	0
6	С	393	0	396	5	0
7	R	22	0	22	0	0
All	All	9371	0	9168	251	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 14.

The worst 5 of 251 close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	$\begin{array}{c} {\rm Interatomic} \\ {\rm distance} \ ({\rm \AA}) \end{array}$	Clash overlap (Å)
3:D:172:THR:CG2	3:D:212:PHE:HE2	1.53	1.21
1:B:198:LEU:HD13	1:B:210:LEU:CD1	1.70	1.21
3:D:172:THR:HG21	3:D:212:PHE:CE2	1.80	1.16
3:D:172:THR:CG2	3:D:212:PHE:CE2	2.31	1.13
3:D:64:VAL:HG11	3:D:68:PHE:CD2	1.87	1.09

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	В	338/340 (99%)	325 (96%)	13 (4%)	0	100 100
2	N	126/128 (98%)	125 (99%)	1 (1%)	0	100 100
3	D	227/246 (92%)	214 (94%)	11 (5%)	2 (1%)	14 26
4	R	246/321 (77%)	240 (98%)	6 (2%)	0	100 100
5	A	220/373~(59%)	217 (99%)	3 (1%)	0	100 100
6	С	50/52~(96%)	47 (94%)	3 (6%)	0	100 100
All	All	1207/1460 (83%)	1168 (97%)	37 (3%)	2 (0%)	45 63

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
3	D	173	TYR
3	D	236	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	Rotameric Outliers		Percentiles		
1	В	$267/283 \; (94\%)$	262 (98%)	5 (2%)	52	71		
2	N	98/106 (92%)	97 (99%)	1 (1%)	73	84		
3	D	191/198 (96%)	190 (100%)	1 (0%)	86	92		
4	R	197/258 (76%)	195 (99%)	2 (1%)	73	84		
5	A	188/332 (57%)	186 (99%)	2 (1%)	70	83		
6	С	41/42 (98%)	41 (100%)	0	100	100		
All	All	982/1219 (81%)	971 (99%)	11 (1%)	69	83		

5 of 11 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
4	R	332	ASN
4	R	352	PHE
5	A	307	ARG
5	A	222	PHE
1	В	325	MET

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (2) such sidechains are listed below:

Mol	Chain	Res	Type
1	В	32	GLN
1	В	340	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)

There are no oligosaccharides in this entry.

5.6 Ligand geometry (i)

1 ligand is 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	Bo	ond leng	ths	В	ond ang	cles
MOI	туре	Chain	nes	Lilik	Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z > 2
7	CAU	R	401	-	22,24,24	0.82	0	31,33,33	0.90	1 (3%)

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.

M	ol	Type	Chain	Res	Link	Chirals	Torsions	Rings
7	,	CAU	R	401	-	-	0/10/10/10	0/3/3/3

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mo	Chain	Res	Type	Atoms	\mathbf{Z}	$Observed(^o)$	$\operatorname{Ideal}({}^{o})$
7	R	401	CAU	C11-C10-C8	-2.16	116.98	120.08

There are no chirality outliers.

There are no torsion outliers.

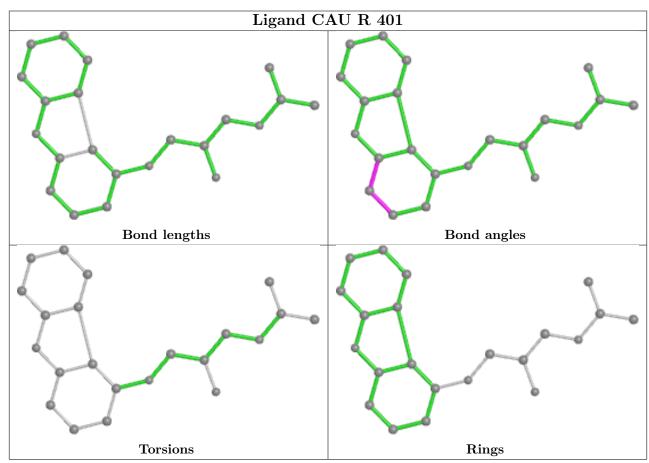
There are no ring outliers.

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 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.



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.

