



# Full wwPDB X-ray Structure Validation Report

Feb 28, 2014 – 03:36 PM GMT

PDB ID : 4MQT  
Title : Structure of active human M2 muscarinic acetylcholine receptor bound to the agonist iperoxo and allosteric modulator LY2119620  
Authors : Kruse, A.C.; Ring, A.M.; Manglik, A.; Hu, J.; Hu, K.; Eitel, K.; Huebner, H.; Pardon, E.; Valant, C.; Sexton, P.M.; Christopoulos, A.; Felder, C.C.; Gmeiner, P.; Steyaert, J.; Weis, W.I.; Garcia, K.C.; Wess, J.; Kobilka, B.K.  
Deposited on : 2013-09-16  
Resolution : 3.70 Å(reported)

This is a full wwPDB validation 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 <http://wwpdb.org/ValidationPDFNotes.html>

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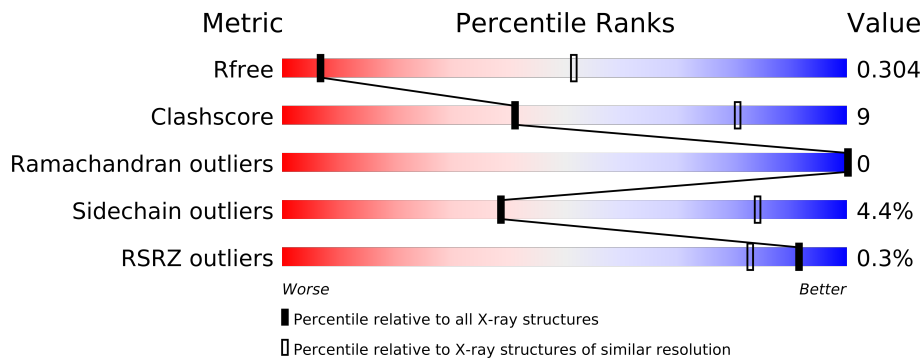
The following versions of software and data (see [references](#)) were used in the production of this report:

MolProbity : 4.02b-467  
Mogul : 1.15 2013  
Xtriage (Phenix) : dev-1323  
EDS : stable22639  
Percentile statistics : 21963  
Refmac : 5.8.0049  
CCP4 : 6.3.0 (Settle)  
Ideal geometry (proteins) : Engh & Huber (2001)  
Ideal geometry (DNA, RNA) : Parkinson et. al. (1996)  
Validation Pipeline (wwPDB-VP) : stable22683

# 1 Overall quality at a glance

The reported resolution of this entry is 3.70 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)	Similar resolution (#Entries, resolution range(Å))
$R_{free}$	66092	1098 (4.00-3.40)
Clashscore	79885	1009 (3.94-3.46)
Ramachandran outliers	78287	1016 (3.98-3.42)
Sidechain outliers	78261	1014 (3.98-3.42)
RSRZ outliers	66119	1099 (4.00-3.40)

The table below summarises the geometric issues observed across the polymeric chains and their fit to the electron density. The red, orange, yellow and green segments on the lower bar indicate the fraction of residues that contain outliers for  $\geq 3$ , 2, 1 and 0 types of geometric quality criteria. The upper red bar (where present) indicates the fraction of residues that have poor fit to the electron density.

Mol	Chain	Length	Quality of chain
1	A	351	
2	B	125	

The following table lists non-polymeric compounds that are outliers for geometric or electron-density-fit criteria:

Mol	Type	Chain	Res	Geometry	Electron density
3	IXO	A	501	-	X

## 2 Entry composition

There are 4 unique types of molecules in this entry. The entry contains 3056 atoms, of which 0 are hydrogen and 0 are deuterium.

In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, 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 Muscarinic acetylcholine receptor M2.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	275	2114	1418	326	354	16	0	0	0

There are 31 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-16	ASP	-	EXPRESSION TAG	UNP P08172
A	-15	TYR	-	EXPRESSION TAG	UNP P08172
A	-14	LYS	-	EXPRESSION TAG	UNP P08172
A	-13	ASP	-	EXPRESSION TAG	UNP P08172
A	-12	ASP	-	EXPRESSION TAG	UNP P08172
A	-11	ASP	-	EXPRESSION TAG	UNP P08172
A	-10	GLU	-	EXPRESSION TAG	UNP P08172
A	-9	ASN	-	EXPRESSION TAG	UNP P08172
A	-8	LEU	-	EXPRESSION TAG	UNP P08172
A	-7	TYR	-	EXPRESSION TAG	UNP P08172
A	-6	PHE	-	EXPRESSION TAG	UNP P08172
A	-5	GLN	-	EXPRESSION TAG	UNP P08172
A	-4	GLY	-	EXPRESSION TAG	UNP P08172
A	-3	LEU	-	EXPRESSION TAG	UNP P08172
A	-2	GLU	-	EXPRESSION TAG	UNP P08172
A	0	ASP	ASN	ENGINEERED MUTATION	UNP P08172
A	1	ASP	ASN	ENGINEERED MUTATION	UNP P08172
A	4	ASP	ASN	ENGINEERED MUTATION	UNP P08172
A	7	ASP	ASN	ENGINEERED MUTATION	UNP P08172
A	17	LEU	-	INSERTION	UNP P08172
A	18	GLU	-	INSERTION	UNP P08172
A	373	THR	ALA	ENGINEERED MUTATION	UNP P08172
A	374	ARG	LYS	ENGINEERED MUTATION	UNP P08172
A	467	HIS	-	EXPRESSION TAG	UNP P08172
A	468	HIS	-	EXPRESSION TAG	UNP P08172
A	469	HIS	-	EXPRESSION TAG	UNP P08172
A	470	HIS	-	EXPRESSION TAG	UNP P08172

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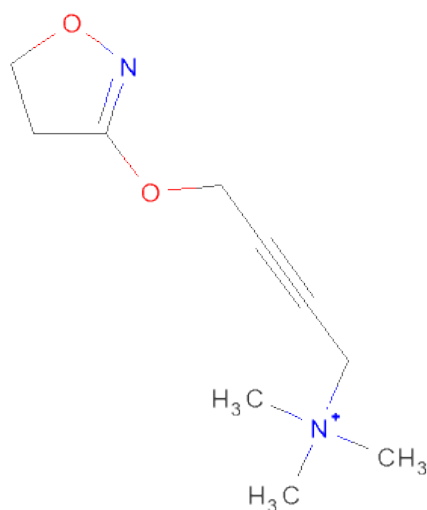
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Chain	Residue	Modelled	Actual	Comment	Reference
A	471	HIS	-	EXPRESSION TAG	UNP P08172
A	472	HIS	-	EXPRESSION TAG	UNP P08172
A	473	HIS	-	EXPRESSION TAG	UNP P08172
A	474	HIS	-	EXPRESSION TAG	UNP P08172

- Molecule 2 is a protein called Nanobody 9-8.

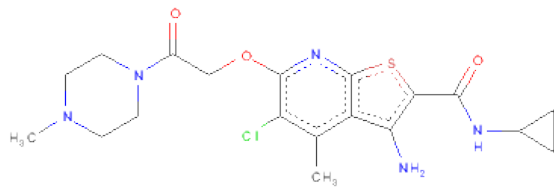
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	B	121	Total	C	N	O	S	0	0	0
			899	557	150	188	4			

- Molecule 3 is 4-(4,5-DIHYDRO-1,2-OXAZOL-3-YLOXY)-N,N,N-TRIMETHYLBUT-2-YN-1-AMINIUM (three-letter code: IXO) (formula:  $C_{10}H_{17}N_2O_2$ ).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
3	A	1	Total	C	N	O	0	0
			14	10	2	2		

- Molecule 4 is 3-AMINO-5-CHLORO-N-CYCLOPROPYL-4-METHYL-6-[2-(4-METHYLPIPERAZIN-1-YL)-2-OXOETHOXY]THIENO[2,3-B]PYRIDINE-2-CARBOXAMIDE (three-letter code: 2CU) (formula:  $C_{19}H_{24}ClN_5O_3S$ ).



Mol	Chain	Residues	Atoms						ZeroOcc	AltConf
			Total	C	Cl	N	O	S		
4	A	1	29	19	1	5	3	1	0	0



## 4 Data and refinement statistics

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	59.03Å 77.40Å 163.82Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	36.00 – 3.70 36.20 – 3.70	Depositor EDS
% Data completeness (in resolution range)	92.9 (36.00-3.70) 88.4 (36.20-3.70)	Depositor EDS
$R_{merge}$	0.20	Depositor
$R_{sym}$	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	2.04 (at 3.66Å)	Xtriage
Refinement program	PHENIX (phenix.refine: dev_1241)	Depositor
R, $R_{free}$	0.250 , 0.301 0.252 , 0.304	Depositor DCC
$R_{free}$ test set	748 reflections (10.00%)	DCC
Wilson B-factor (Å <sup>2</sup> )	80.7	Xtriage
Anisotropy	0.481	Xtriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.24 , 54.9	EDS
Estimated twinning fraction	No twinning to report.	Xtriage
L-test for twinning	$\langle  L  \rangle = 0.41$ , $\langle L^2 \rangle = 0.23$	Xtriage
Outliers	0 of 7871 reflections	Xtriage
$F_o, F_c$ correlation	0.87	EDS
Total number of atoms	3056	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	110.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 7.45% of the height of the origin peak. No significant pseudotranslation is detected.*

<sup>1</sup>Intensities estimated from amplitudes.

## 5 Model quality

### 5.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: 2CU, IXO

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	0.25	0/2174	0.44	0/2992
2	B	0.25	0/919	0.43	0/1252
All	All	0.25	0/3093	0.44	0/4244

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no planarity outliers.

### 5.2 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 hydrogens added by MolProbity. The Clashes column lists the number of clashes within the asymmetric unit, and the number in parentheses is this value normalized per 1000 atoms of the molecule in the chain. The Symm-Clashes column gives symmetry related clashes, in the same way as for the Clashes column.

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	2114	0	2112	37	0
2	B	899	0	795	19	0
3	A	14	0	17	0	0
4	A	29	0	24	4	0
All	All	3056	0	2948	55	0

Clashscore is defined as the number of clashes calculated for the entry per 1000 atoms (including hydrogens) of the entry. The overall clashscore for this entry is 9.

All (55) close contacts within the same asymmetric unit are listed below.



Atom-1	Atom-2	Distance(Å)	Clash(Å)
2:B:33:ASP:O	2:B:57:SER:OG	2.04	0.74
1:A:419:ASN:HB3	4:A:502:2CU:H25	1.72	0.71
1:A:100:LEU:HB3	1:A:178:ILE:HD11	1.76	0.68
1:A:83:TYR:HE1	1:A:176:CYS:HB3	1.60	0.66
1:A:118:SER:HB3	1:A:205:LEU:HD11	1.80	0.63
1:A:402:PRO:HG2	1:A:428:LEU:HD23	1.82	0.60
2:B:31:ASN:O	2:B:34:ASP:N	2.32	0.60
4:A:502:2CU:H1	4:A:502:2CU:H19	1.66	0.59
1:A:446:THR:OG1	2:B:108:ASP:OD1	2.20	0.59
1:A:410:ASN:HB2	1:A:417:ILE:HD12	1.84	0.58
2:B:27:PHE:CD2	2:B:27:PHE:N	2.72	0.58
1:A:34:SER:OG	1:A:73:GLY:O	2.21	0.56
1:A:76:SER:HA	1:A:99:TRP:HE1	1.72	0.54
1:A:91:LEU:HB3	1:A:95:VAL:HG11	1.90	0.52
2:B:103:THR:OG1	2:B:108:ASP:HB2	2.09	0.52
1:A:129:LEU:HD21	2:B:53:CYS:HB3	1.91	0.51
1:A:71:ILE:HG21	1:A:106:VAL:HG11	1.93	0.50
1:A:451:PHE:HA	1:A:454:LEU:HD23	1.94	0.50
2:B:12:VAL:HG13	2:B:16:ASP:HB2	1.94	0.49
1:A:210:SER:HB3	1:A:389:ILE:HD12	1.93	0.49
1:A:79:LEU:HD23	1:A:89:TRP:HH2	1.76	0.49
1:A:128:PRO:HG2	2:B:62:ILE:HG21	1.95	0.47
1:A:403:TYR:HB3	1:A:429:CYS:SG	2.55	0.46
1:A:188:PHE:CE1	1:A:408:LEU:HG	2.50	0.46
1:A:162:TRP:HB3	1:A:179:GLN:HG3	1.99	0.45
1:A:74:VAL:O	1:A:78:ASN:ND2	2.33	0.45
1:A:33:LEU:HD21	1:A:431:ILE:HG22	1.97	0.45
2:B:30:ASP:HB3	2:B:35:TYR:CE2	2.52	0.45
1:A:380:SER:O	1:A:384:LYS:HG2	2.17	0.45
2:B:30:ASP:HB3	2:B:35:TYR:HE2	1.81	0.44
1:A:414:ALA:HB1	4:A:502:2CU:H11	1.99	0.44
1:A:197:LEU:HB3	1:A:198:PRO:HD3	1.99	0.44
1:A:406:MET:HE1	1:A:422:TRP:HA	2.00	0.44
1:A:421:VAL:HA	1:A:424:ILE:HD12	1.99	0.44
1:A:192:ILE:HA	1:A:196:TYR:HD2	1.82	0.43
2:B:75:SER:HB3	2:B:82:VAL:HG12	2.00	0.43
1:A:125:VAL:HG21	1:A:209:ILE:HG23	2.00	0.42
1:A:102:LEU:O	1:A:106:VAL:HG22	2.19	0.42
2:B:63:TYR:HE1	2:B:73:ILE:HG22	1.84	0.42
2:B:4:LEU:O	2:B:113:GLN:NE2	2.52	0.42
1:A:38:ILE:HG22	1:A:70:LEU:HD12	2.02	0.42
1:A:116:ILE:HG12	1:A:143:MET:HB3	2.01	0.42
2:B:101:ALA:O	2:B:109:GLU:HA	2.20	0.42

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Atom-1	Atom-2	Distance(Å)	Clash(Å)
2:B:6:GLU:HB2	2:B:115:THR:HG23	2.02	0.41
2:B:56:PRO:HB2	2:B:77:ASN:HD21	1.85	0.41
1:A:91:LEU:HB3	1:A:95:VAL:CG1	2.51	0.41
2:B:53:CYS:SG	2:B:62:ILE:HB	2.59	0.41
1:A:70:LEU:O	1:A:74:VAL:HG12	2.21	0.41
1:A:406:MET:HE1	1:A:421:VAL:O	2.20	0.41
1:A:432:ASN:HA	1:A:435:ILE:HG12	2.03	0.41
1:A:43:LEU:HD11	1:A:455:LEU:HD21	2.03	0.41
2:B:27:PHE:HD2	2:B:27:PHE:H	1.70	0.40
4:A:502:2CU:H4	4:A:502:2CU:H15	1.93	0.40
1:A:43:LEU:CD1	1:A:455:LEU:HD21	2.51	0.40
2:B:86:MET:HB2	2:B:89:LEU:HD21	2.03	0.40

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 X-ray entries followed by that with respect to entries of similar resolution. 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	271/351 (77%)	262 (97%)	9 (3%)	0	100	100
2	B	119/125 (95%)	112 (94%)	7 (6%)	0	100	100
All	All	390/476 (82%)	374 (96%)	16 (4%)	0	100	100

There are no Ramachandran outliers to report.

### 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 X-ray entries followed by that with respect to entries of similar resolution. 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	223/312 (72%)	212 (95%)	11 (5%)	35	82

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
2	B	92/101 (91%)	89 (97%)	3 (3%)	50	89
All	All	315/413 (76%)	301 (96%)	14 (4%)	39	84

All (14) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	24	VAL
1	A	50	VAL
1	A	108	ASN
1	A	113	ASN
1	A	179	GLN
1	A	180	PHE
1	A	183	ASN
1	A	211	ARG
1	A	407	VAL
1	A	411	THR
1	A	426	TYR
2	B	12	VAL
2	B	27	PHE
2	B	113	GLN

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (3) such sidechains are listed below:

Mol	Chain	Res	Type
2	B	77	ASN
2	B	80	ASN
2	B	113	GLN

### 5.3.3 RNA ⓘ

There are no RNA chains 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 ⓘ

There are no carbohydrates in this entry.

## 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	$\# Z  > 2$
3	IXO	A	501	-	14,14,14	7.14	10 (71%)	18,18,18	2.11	5 (27%)
4	2CU	A	502	-	32,32,32	2.78	12 (37%)	40,47,47	5.41	12 (30%)

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	IXO	A	501	-	-	2/9/16/16	0/1/1/1
4	2CU	A	502	-	-	0/15/29/29	0/1/4/4

All (22) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	A	501	IXO	C10-N11	24.27	1.41	1.28
4	A	502	2CU	C01-N02	-8.47	1.24	1.46
4	A	502	2CU	C24-N26	5.07	1.45	1.34
3	A	501	IXO	O12-N11	-5.06	1.37	1.43
4	A	502	2CU	C08-N05	5.06	1.47	1.35
4	A	502	2CU	C06-N05	-4.85	1.38	1.47
3	A	501	IXO	C05-C06	4.69	1.51	1.46
4	A	502	2CU	C04-N05	-4.41	1.39	1.47
3	A	501	IXO	C05-N02	-4.29	1.45	1.51
4	A	502	2CU	C18-N19	4.20	1.44	1.36
4	A	502	2CU	C22-S21	-4.18	1.68	1.74
3	A	501	IXO	C14-C10	-4.05	1.41	1.49
4	A	502	2CU	O11-C12	3.05	1.39	1.34
4	A	502	2CU	C18-C17	3.00	1.52	1.47
3	A	501	IXO	C07-C06	2.88	1.29	1.19
3	A	501	IXO	C14-C13	-2.83	1.45	1.51

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Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	A	501	IXO	O09-C10	2.81	1.37	1.34
4	A	502	2CU	C07-N02	-2.36	1.40	1.46
3	A	501	IXO	C03-N02	-2.34	1.43	1.50
4	A	502	2CU	C03-N02	-2.33	1.40	1.46
3	A	501	IXO	O09-C08	-2.13	1.42	1.45
4	A	502	2CU	C12-N23	2.02	1.35	1.30

All (17) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
4	A	502	2CU	C20-S21-C22	29.52	100.44	92.06
4	A	502	2CU	C17-C22-S21	-10.80	106.77	113.03
4	A	502	2CU	S21-C22-N23	6.22	131.31	124.29
4	A	502	2CU	C12-C13-CL1	-5.21	117.30	121.03
3	A	501	IXO	C08-O09-C10	5.01	121.56	115.21
4	A	502	2CU	C13-C12-N23	-4.95	119.03	123.08
4	A	502	2CU	C18-C17-C22	4.53	114.64	107.91
3	A	501	IXO	O09-C10-N11	4.45	125.11	123.94
3	A	501	IXO	C06-C05-N02	3.29	117.19	112.17
3	A	501	IXO	O09-C08-C07	3.18	117.70	109.39
4	A	502	2CU	O11-C12-C13	3.03	120.32	117.62
4	A	502	2CU	C20-C24-N26	2.96	119.87	115.98
4	A	502	2CU	C12-N23-C22	2.60	121.94	115.31
3	A	501	IXO	C14-C10-N11	-2.51	107.93	115.21
4	A	502	2CU	O11-C10-C08	2.50	117.11	109.69
4	A	502	2CU	C10-C08-N05	2.35	123.17	116.89
4	A	502	2CU	C28-C27-N26	-2.24	115.67	118.23

There are no chirality outliers.

All (2) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	A	501	IXO	C07-C06-C05-N02
3	A	501	IXO	C05-C06-C07-C08

There are no ring outliers.

## 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.

## 6 Fit of model and data ⓘ

### 6.1 Protein, DNA and RNA chains ⓘ

In the following table, the column labelled ‘#RSRZ> 2’ contains the number (and percentage) of RSRZ outliers, followed by percent RSRZ outliers for the chain as percentile scores relative to all X-ray entries and entries of similar resolution. The OWAB column contains the minimum, median, 95<sup>th</sup> percentile and maximum values of the occupancy-weighted average B-factor per residue. The column labelled ‘Q< 0.9’ lists the number of (and percentage) of residues with an average occupancy less than 0.9.

Mol	Chain	Analysed	<RSRZ>	#RSRZ>2	OWAB(Å <sup>2</sup> )	Q<0.9
1	A	275/351 (78%)	-0.28	1 (0%) 90 78	62, 98, 146, 187	0
2	B	121/125 (96%)	0.01	0 100 100	83, 125, 164, 193	0
All	All	396/476 (83%)	-0.19	1 (0%) 91 83	62, 108, 151, 193	0

All (1) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	379	PRO	2.3

### 6.2 Non-standard residues in protein, DNA, RNA chains ⓘ

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

### 6.3 Carbohydrates ⓘ

There are no carbohydrates in this entry.

### 6.4 Ligands ⓘ

In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. LLDF column lists the quality of electron density of the group with respect to its neighbouring residues in protein, DNA or RNA chains. The B-factors column lists the minimum, median, 95<sup>th</sup> percentile and maximum values of B factors of atoms in the group. The column labelled ‘Q< 0.9’ lists the number of atoms with occupancy less than 0.9.

Mol	Type	Chain	Res	Atoms	RSR	LLDF	B-factors(Å <sup>2</sup> )	Q<0.9
3	IXO	A	501	14/14	0.29	3.80	83,104,131,136	0
4	2CU	A	502	29/29	0.25	0.94	75,110,164,174	0

## 6.5 Other polymers ⓘ

There are no such residues in this entry.