



Full wwPDB X-ray Structure Validation Report

Sep 5, 2014 – 03:10 AM EDT

PDB ID : 4PHM
Title : The Structural Basis of Differential Inhibition of Human Calpain by Indole and Phenyl alpha-MercaptoacrylicAcids
Authors : Rizkallah, P.J.; Allemann, R.K.; Adams, S.E.; Miller, D.J.; Hallett, M.B.
Deposited on : 2014-05-06
Resolution : 2.03 Å(reported)

This is a full wwPDB validation report for a publicly released PDB entry.
We welcome your comments at validation@mail.wwpdb.org
A user guide is available at <http://wwpdb.org/ValidationPDFNotes.html>

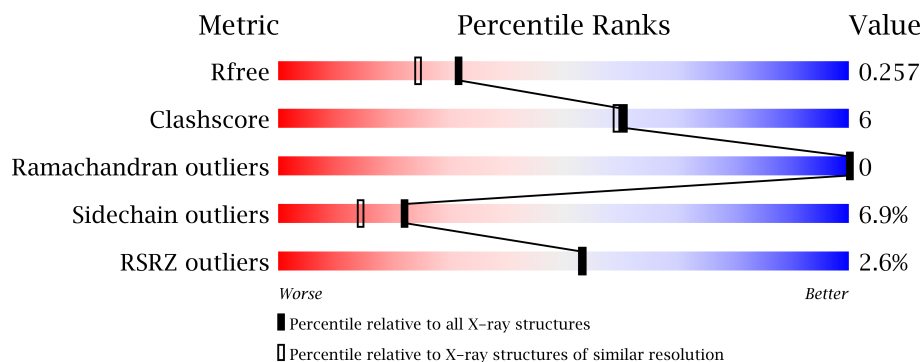
The following versions of software and data (see [references](#)) were used in the production of this report:

MolProbity : 4.02b-467
Mogul : 1.16 November 2013
Xtriage (Phenix) : dev-1439
EDS : stable23489
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) : stable23489

1 Overall quality at a glance

The reported resolution of this entry is 2.03 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	6003 (2.04-2.00)
Clashscore	79885	7467 (2.04-2.00)
Ramachandran outliers	78287	7370 (2.04-2.00)
Sidechain outliers	78261	7368 (2.04-2.00)
RSRZ outliers	66119	6006 (2.04-2.00)

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 ≥ 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	173	
1	B	173	

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

Mol	Type	Chain	Res	Geometry	Electron density
2	2UD	A	301[A]	-	X
2	2UD	A	301[B]	-	X
2	2UD	B	301	-	X

2 Entry composition i

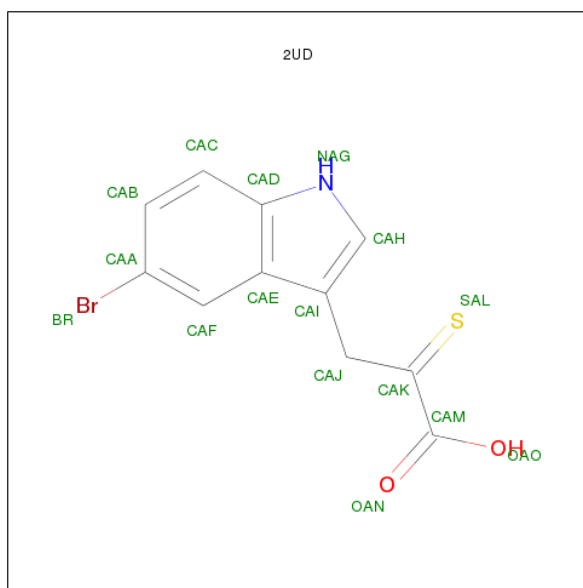
There are 4 unique types of molecules in this entry. The entry contains 3012 atoms, of which 0 are hydrogen and 0 are deuterium.

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 Calpain small subunit 1.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	173	Total	C	N	O	S	0	0	0
			1403	883	240	269	11			
1	B	173	Total	C	N	O	S	0	0	0
			1403	883	240	269	11			

- Molecule 2 is 3-(5-bromo-1H-indol-3-yl)-2-thioxopropanoicacid (three-letter code: 2UD) (formula: C₁₁H₈BrNO₂S).



Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
2	A	1	Total	Br	C	N	O	S	0
			32	2	22	2	4	2	
2	B	1	Total	Br	C	N	O	S	0
			16	1	11	1	2	1	

- Molecule 3 is CALCIUM ION (three-letter code: CA) (formula: Ca).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
3	B	4	Total 4	Ca 4	0	0
3	A	4	Total 4	Ca 4	0	0

- Molecule 4 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
4	A	57	Total 57	O 57	0	0
4	B	93	Total 93	O 93	0	0

3 Residue-property plots

These plots are drawn for all protein, RNA and DNA chains in the entry. The first graphic for a chain summarises the proportions of errors displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and electron density. 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. A red dot above a residue indicates a poor fit to the electron density ($RSRZ > 2$). 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.

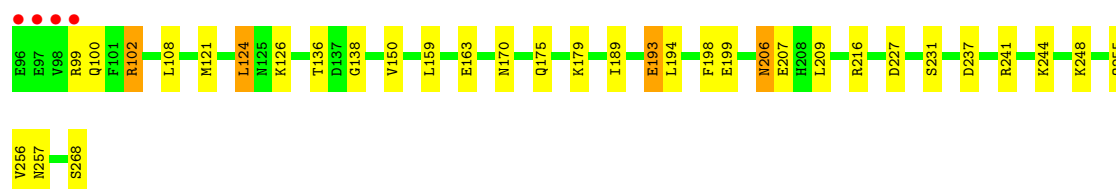
- Molecule 1: Calpain small subunit 1

Chain A: 



- Molecule 1: Calpain small subunit 1

Chain B: 



4 Data and refinement statistics

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α , β , γ	49.41 Å 79.34 Å 57.26 Å 90.00° 91.23° 90.00°	Depositor
Resolution (Å)	41.93 – 2.03 41.93 – 2.03	Depositor EDS
% Data completeness (in resolution range)	94.9 (41.93-2.03) 94.9 (41.93-2.03)	Depositor EDS
R_{merge}	0.04	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	2.01 (at 2.03 Å)	Xtriage
Refinement program	REFMAC 5.8.0049	Depositor
R, R_{free}	0.197 , 0.251 0.208 , 0.257	Depositor DCC
R_{free} test set	1360 reflections (5.27%)	DCC
Wilson B-factor (Å ²)	33.2	Xtriage
Anisotropy	0.121	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.33 , 37.1	EDS
Estimated twinning fraction	0.043 for h,-k,-l	Xtriage
L-test for twinning	$\langle L \rangle = 0.49$, $\langle L^2 \rangle = 0.32$	Xtriage
Outliers	5 of 27171 reflections (0.018%)	Xtriage
F_o, F_c correlation	0.94	EDS
Total number of atoms	3012	wwPDB-VP
Average B, all atoms (Å ²)	44.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 6.50% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities 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: CA, 2UD

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	0.89	1/1430 (0.1%)	0.95	3/1923 (0.2%)
1	B	1.04	2/1430 (0.1%)	1.09	4/1923 (0.2%)
All	All	0.97	3/2860 (0.1%)	1.02	7/3846 (0.2%)

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	1

All (3) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	267	TYR	CE1-CZ	6.86	1.47	1.38
1	B	231	SER	CB-OG	-6.80	1.33	1.42
1	B	193	GLU	CD-OE2	-6.15	1.18	1.25

All (7) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	B	121	MET	CG-SD-CE	5.38	108.81	100.20
1	A	104	LEU	CA-CB-CG	5.34	127.58	115.30
1	B	237	ASP	CB-CG-OD2	5.25	123.03	118.30
1	A	215	ARG	NE-CZ-NH2	-5.18	117.71	120.30
1	B	124	LEU	CB-CG-CD1	5.17	119.78	111.00
1	B	241	ARG	NE-CZ-NH1	5.10	122.85	120.30
1	A	219	ASP	CB-CG-OD1	5.01	122.81	118.30

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	110	GLY	Peptide

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	1403	0	1348	15	0
1	B	1403	0	1348	19	0
2	A	32	0	0	2	0
2	B	16	0	0	1	0
3	A	4	0	0	0	0
3	B	4	0	0	0	0
4	A	57	0	0	3	0
4	B	93	0	0	1	0
All	All	3012	0	2696	33	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 6.

All (33) close contacts within the same asymmetric unit are listed below.

Atom-1	Atom-2	Distance(Å)	Clash(Å)
1:A:267:TYR:O	4:A:452:HOH:O	1.67	1.13
1:B:136:THR:HG22	1:B:138:GLY:H	1.36	0.89
1:B:102:ARG:HH11	1:B:102:ARG:HG3	1.37	0.87
1:A:253:GLN:OE1	1:B:255:GLN:OE1	1.94	0.86
1:B:136:THR:HG23	1:B:227:ASP:OD1	1.81	0.80
1:B:102:ARG:HH11	1:B:102:ARG:CG	2.02	0.72
1:B:136:THR:HG22	1:B:138:GLY:N	2.05	0.71
1:B:136:THR:CG2	1:B:138:GLY:H	2.04	0.71
1:B:199:GLU:OE1	4:B:457:HOH:O	2.09	0.69
1:A:129:THR:HB	4:A:430:HOH:O	1.94	0.67
1:A:268:SER:O	4:A:428:HOH:O	2.16	0.61
1:B:136:THR:CG2	1:B:227:ASP:OD1	2.52	0.56

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Atom-1	Atom-2	Distance(Å)	Clash(Å)
1:B:175:GLN:HE21	1:B:179:LYS:HE3	1.71	0.56
1:A:99:ARG:NH1	1:A:99:ARG:HB3	2.22	0.54
1:B:194:LEU:HD11	1:B:198:PHE:CE1	2.45	0.51
1:B:102:ARG:NH1	1:B:102:ARG:HG3	2.15	0.50
1:B:206:ASN:C	1:B:206:ASN:HD22	2.13	0.50
2:A:301[A]:2UD:CAH	2:A:301[A]:2UD:OAN	2.64	0.46
2:B:301:2UD:SAL	2:B:301:2UD:CAH	3.03	0.46
1:A:150:VAL:HG13	1:A:238:ALA:CB	2.48	0.44
1:A:99:ARG:HB3	1:A:99:ARG:HH11	1.82	0.44
1:B:189:ILE:HA	1:B:193:GLU:OE1	2.18	0.44
1:B:100:GLN:HA	1:B:100:GLN:OE1	2.17	0.43
1:A:182:ASP:O	1:A:185:ARG:NH1	2.51	0.43
1:A:96:GLU:HA	1:A:99:ARG:HG3	2.01	0.42
1:B:159:LEU:HA	1:B:163:GLU:OE1	2.18	0.42
1:A:267:TYR:CD1	1:B:216:ARG:HB2	2.54	0.42
1:A:114:GLU:OE1	1:A:158:LYS:HB2	2.20	0.42
1:A:172:LYS:HD2	2:A:301[A]:2UD:CAH	2.50	0.42
1:B:170:ASN:HD22	1:B:170:ASN:HA	1.63	0.42
1:A:168:TRP:O	1:A:172:LYS:HG3	2.20	0.41
1:A:100:GLN:HE21	1:A:100:GLN:HB2	1.58	0.41
1:A:267:TYR:CE1	1:B:216:ARG:HB2	2.57	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	171/173 (99%)	170 (99%)	1 (1%)	0	100	100
1	B	171/173 (99%)	169 (99%)	2 (1%)	0	100	100
All	All	342/346 (99%)	339 (99%)	3 (1%)	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	152/152 (100%)	145 (95%)	7 (5%)	37	30
1	B	152/152 (100%)	138 (91%)	14 (9%)	13	7
All	All	304/304 (100%)	283 (93%)	21 (7%)	22	14

All (21) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	96	GLU
1	A	100	GLN
1	A	104	LEU
1	A	108	LEU
1	A	124	LEU
1	A	206	ASN
1	A	259	GLN
1	B	99	ARG
1	B	102	ARG
1	B	108	LEU
1	B	124	LEU
1	B	126	LYS
1	B	150	VAL
1	B	206	ASN
1	B	207	GLU
1	B	209	LEU
1	B	244	LYS
1	B	248	LYS
1	B	256	VAL
1	B	257	ASN
1	B	268	SER

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (10) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	100	GLN
1	A	170	ASN
1	A	204	HIS

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Mol	Chain	Res	Type
1	A	206	ASN
1	A	253	GLN
1	A	259	GLN
1	B	170	ASN
1	B	175	GLN
1	B	206	ASN
1	B	253	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 ⓘ

Of 11 ligands modelled in this entry, 8 are monoatomic - leaving 3 for Mogul analysis.

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	$\# Z > 2$
2	2UD	A	301[A]	-	17,17,17	2.73	9 (52%)	24,24,24	2.17	5 (20%)
2	2UD	A	301[B]	-	17,17,17	2.90	10 (58%)	24,24,24	2.13	8 (33%)
2	2UD	B	301	-	17,17,17	3.15	10 (58%)	24,24,24	2.41	5 (20%)

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
2	2UD	A	301[A]	-	-	1/6/8/8	0/2/2/2
2	2UD	A	301[B]	-	-	1/6/8/8	0/2/2/2
2	2UD	B	301	-	-	1/6/8/8	0/2/2/2

All (29) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	B	301	2UD	CAK-SAL	6.66	1.91	1.64
2	B	301	2UD	CAJ-CAI	-6.59	1.32	1.52
2	A	301[A]	2UD	CAJ-CAI	-5.98	1.34	1.52
2	A	301[B]	2UD	CAJ-CAK	5.82	1.56	1.50
2	A	301[B]	2UD	CAJ-CAI	-5.57	1.35	1.52
2	B	301	2UD	CAE-CAD	-4.03	1.34	1.41
2	A	301[A]	2UD	CAE-CAD	-3.91	1.34	1.41
2	A	301[A]	2UD	BR-CAA	3.85	1.99	1.90
2	A	301[B]	2UD	CAE-CAD	-3.73	1.34	1.41
2	B	301	2UD	CAF-CAE	-3.70	1.34	1.42
2	A	301[A]	2UD	CAF-CAE	-3.52	1.35	1.42
2	B	301	2UD	CAC-CAD	-3.39	1.33	1.40
2	A	301[A]	2UD	CAC-CAD	-3.31	1.33	1.40
2	A	301[B]	2UD	CAF-CAE	-3.22	1.35	1.42
2	A	301[B]	2UD	CAC-CAD	-3.22	1.34	1.40
2	A	301[B]	2UD	BR-CAA	3.12	1.97	1.90
2	A	301[A]	2UD	CAJ-CAK	2.97	1.53	1.50
2	B	301	2UD	CAI-CAE	-2.87	1.33	1.42
2	B	301	2UD	CAJ-CAK	2.79	1.53	1.50
2	A	301[A]	2UD	CAF-CAA	2.54	1.40	1.36
2	A	301[B]	2UD	CAF-CAA	2.48	1.40	1.36
2	A	301[B]	2UD	CAI-CAE	-2.42	1.35	1.42
2	B	301	2UD	CAH-NAG	-2.32	1.33	1.37
2	A	301[A]	2UD	CAI-CAE	-2.29	1.35	1.42
2	B	301	2UD	CAF-CAA	2.27	1.40	1.36
2	A	301[B]	2UD	CAH-CAI	-2.23	1.33	1.37
2	B	301	2UD	CAH-CAI	-2.20	1.33	1.37
2	A	301[A]	2UD	CAH-CAI	-2.15	1.33	1.37
2	A	301[B]	2UD	CAH-NAG	-2.13	1.33	1.37

All (18) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	B	301	2UD	CAM-CAK-SAL	9.36	120.39	114.69
2	A	301[A]	2UD	CAM-CAK-SAL	6.89	118.89	114.69
2	A	301[A]	2UD	CAB-CAA-CAF	-5.03	117.38	121.95
2	A	301[B]	2UD	CAB-CAA-CAF	-4.82	117.57	121.95
2	A	301[B]	2UD	CAI-CAJ-CAK	4.51	127.28	114.89
2	A	301[B]	2UD	CAJ-CAK-CAM	4.05	126.30	118.00
2	B	301	2UD	CAJ-CAK-SAL	3.59	124.65	119.71
2	A	301[B]	2UD	CAM-CAK-SAL	3.48	116.81	114.69
2	A	301[A]	2UD	CAI-CAJ-CAK	3.27	123.87	114.89
2	B	301	2UD	CAI-CAJ-CAK	3.04	123.23	114.89
2	A	301[B]	2UD	BR-CAA-CAF	2.65	123.04	119.78
2	A	301[A]	2UD	CAJ-CAK-CAM	2.58	123.30	118.00
2	A	301[B]	2UD	OAD-CAM-CAK	2.49	123.25	116.15
2	B	301	2UD	CAB-CAA-CAF	-2.42	119.75	121.95
2	A	301[A]	2UD	CAH-CAI-CAE	2.10	109.97	107.32
2	A	301[B]	2UD	CAJ-CAK-SAL	-2.09	116.83	119.71
2	B	301	2UD	CAH-CAI-CAE	2.07	109.93	107.32
2	A	301[B]	2UD	CAH-CAI-CAE	2.04	109.90	107.32

There are no chirality outliers.

All (3) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	B	301	2UD	CAI-CAJ-CAK-SAL
2	A	301[A]	2UD	CAI-CAJ-CAK-SAL
2	A	301[B]	2UD	CAI-CAJ-CAK-SAL

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, 95th 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 ²)	Q<0.9
1	A	173/173 (100%)	-0.07	5 (2%) 49 49	28, 46, 69, 97	0
1	B	173/173 (100%)	-0.06	4 (2%) 57 58	26, 37, 60, 104	0
All	All	346/346 (100%)	-0.06	9 (2%) 53 53	26, 42, 68, 104	0

All (9) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	B	98	VAL	4.4
1	B	96	GLU	4.1
1	B	97	GLU	4.0
1	A	97	GLU	3.5
1	A	96	GLU	2.9
1	A	98	VAL	2.8
1	B	99	ARG	2.5
1	A	248	LYS	2.3
1	A	99	ARG	2.1

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, 95th 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 ²)	Q<0.9
2	2UD	B	301	16/16	0.18	7.66	78,80,85,91	0
2	2UD	A	301[B]	16/16	0.32	6.69	73,76,80,80	16
2	2UD	A	301[A]	16/16	0.32	6.04	72,78,81,84	16
3	CA	B	304	1/1	0.13	1.89	40,40,40,40	0
3	CA	B	303	1/1	0.10	0.65	37,37,37,37	0
3	CA	B	302	1/1	0.09	-0.72	36,36,36,36	0
3	CA	A	305	1/1	0.06	-1.26	37,37,37,37	0
3	CA	A	304	1/1	0.03	-1.70	52,52,52,52	0
3	CA	A	302	1/1	0.03	-1.77	43,43,43,43	0
3	CA	B	305	1/1	0.09	-1.77	30,30,30,30	0
3	CA	A	303	1/1	0.04	-2.48	45,45,45,45	0

6.5 Other polymers ⓘ

There are no such residues in this entry.