



Full wwPDB X-ray Structure Validation Report ⓘ

May 25, 2020 – 10:56 am BST

PDB ID : 3U0K
Title : Crystal Structure of the genetically encoded calcium indicator RCaMP
Authors : Akerboom, J.; Looger, L.L.; Schreiter, E.R.
Deposited on : 2011-09-28
Resolution : 2.10 Å(reported)

This is a Full wwPDB X-ray Structure Validation 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/XrayValidationReportHelp>

with specific help available everywhere you see the ⓘ symbol.

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

MolProbity : 4.02b-467
Mogul : 1.8.5 (274361), CSD as541be (2020)
Xtriage (Phenix) : 1.13
EDS : 2.11
Percentile statistics : 20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac : 5.8.0158
CCP4 : 7.0.044 (Gargrove)
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : 2.11

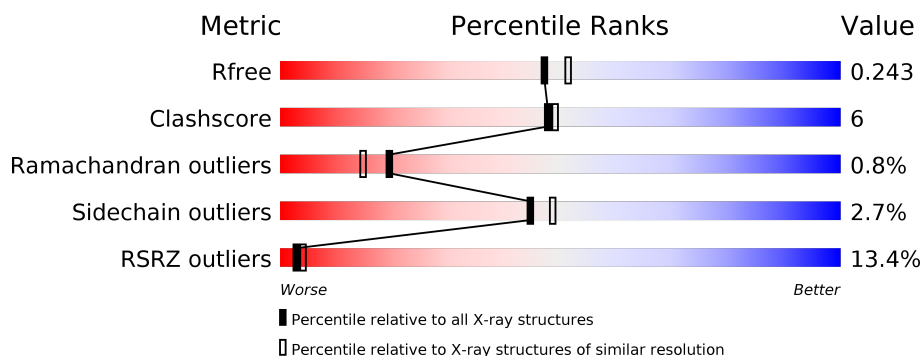
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 2.10 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}	130704	5197 (2.10-2.10)
Clashscore	141614	5710 (2.10-2.10)
Ramachandran outliers	138981	5647 (2.10-2.10)
Sidechain outliers	138945	5648 (2.10-2.10)
RSRZ outliers	127900	5083 (2.10-2.10)

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 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 $\leq 5\%$. The upper red bar (where present) indicates the fraction of residues that have poor fit to the electron density. The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	440	<div> <div>12%</div> <div>75%</div> <div>15%</div> <div>10%</div> </div>

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
2	ACT	A	443	-	-	X	-

2 Entry composition [i](#)

There are 4 unique types of molecules in this entry. The entry contains 3302 atoms, of which 0 are hydrogens and 0 are deuteriums.

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 RCaMP.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	398	3156	1974	542	612	28	0	6	0

- Molecule 2 is ACETATE ION (three-letter code: ACT) (formula: $C_2H_3O_2$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
			Total	C	O		
2	A	1	4	2	2	0	0

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

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

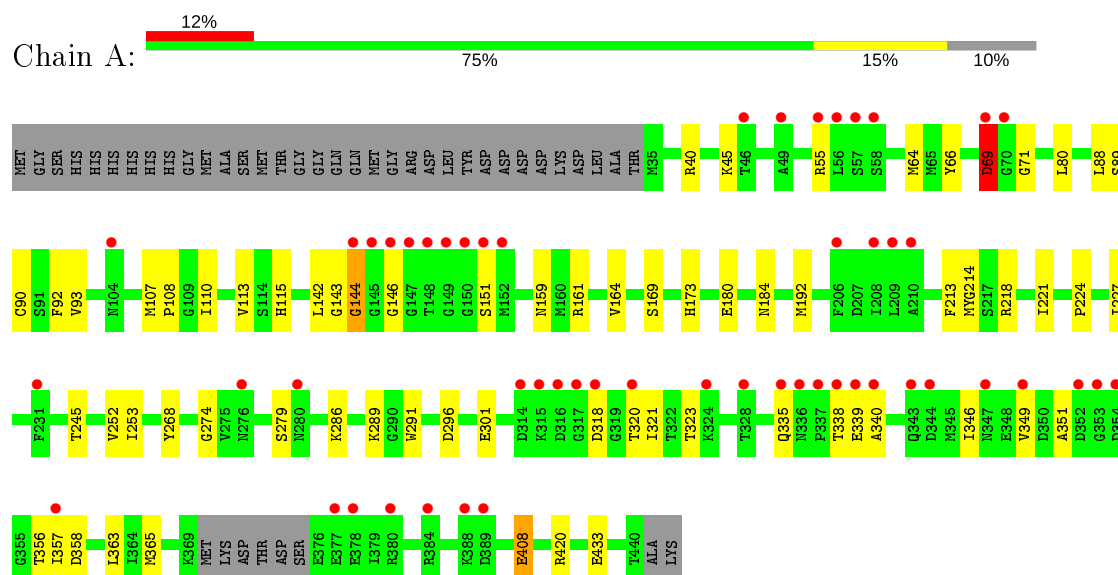
- Molecule 4 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
4	A	138	Total 138	O 138	0	0

3 Residue-property plots [i](#)

These plots are drawn for all protein, RNA and DNA 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 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 ($\text{RSRZ} > 2$). 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: RCaMP



4 Data and refinement statistics

Property	Value	Source
Space group	P 32 2 1	Depositor
Cell constants a, b, c, α , β , γ	75.92Å 75.92Å 123.16Å 90.00° 90.00° 120.00°	Depositor
Resolution (Å)	21.58 – 2.10 21.58 – 2.10	Depositor EDS
% Data completeness (in resolution range)	99.8 (21.58-2.10) 99.9 (21.58-2.10)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	5.45 (at 2.09Å)	Xtriage
Refinement program	REFMAC 5.6.0111	Depositor
R, R_{free}	0.189 , 0.239 0.194 , 0.243	Depositor DCC
R_{free} test set	1253 reflections (5.10%)	wwPDB-VP
Wilson B-factor (Å ²)	29.7	Xtriage
Anisotropy	0.633	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.40 , 53.7	EDS
L-test for twinning ²	$\langle L \rangle = 0.49$, $\langle L^2 \rangle = 0.32$	Xtriage
Estimated twinning fraction	0.033 for -h,-k,l	Xtriage
F_o, F_c correlation	0.95	EDS
Total number of atoms	3302	wwPDB-VP
Average B, all atoms (Å ²)	41.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.82% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical values of $\langle |L| \rangle$, $\langle L^2 \rangle$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

5 Model quality

5.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: CRK, CA, NFA, ACT

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	1.07	2/3193 (0.1%)	0.94	5/4286 (0.1%)

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 (2) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	113	VAL	CB-CG2	6.67	1.66	1.52
1	A	433	GLU	CD-OE1	6.15	1.32	1.25

All (5) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	40	ARG	NE-CZ-NH2	-10.60	115.00	120.30
1	A	161	ARG	NE-CZ-NH1	5.94	123.27	120.30
1	A	88	LEU	CB-CG-CD1	-5.21	102.15	111.00
1	A	296	ASP	CB-CG-OD2	5.14	122.92	118.30
1	A	253	ILE	CB-CA-C	-5.04	101.51	111.60

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	69	ASP	Peptide

5.2 Too-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 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	3156	0	3073	38	0
2	A	4	0	3	4	0
3	A	4	0	0	0	0
4	A	138	0	0	2	0
All	All	3302	0	3076	38	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 6.

All (38) 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:A:93:VAL:HG21	1:A:351:ALA:HB1	1.35	1.04
1:A:93:VAL:CG2	1:A:351:ALA:HB1	1.88	1.03
1:A:318:ASP:OD2	1:A:320:THR:HG22	1.64	0.95
1:A:286:LYS:NZ	4:A:575:HOH:O	2.00	0.90
1:A:66:TYR:CE1	1:A:110:ILE:HD11	2.15	0.82
1:A:323:THR:HG22	1:A:346:ILE:HD12	1.65	0.76
1:A:142:LEU:HD22	1:A:146:GLY:O	1.90	0.71
1:A:66:TYR:CD1	1:A:110:ILE:HG12	2.27	0.70
1:A:93:VAL:HG22	1:A:351:ALA:HB1	1.79	0.65
1:A:115:HIS:HE1	2:A:443:ACT:H2	1.62	0.64
1:A:93:VAL:HG21	1:A:351:ALA:CB	2.21	0.64
1:A:338:THR:OG1	1:A:340:ALA:HB3	1.99	0.61
1:A:66:TYR:CE1	1:A:110:ILE:CD1	2.85	0.58
1:A:289:LYS:NZ	4:A:541:HOH:O	2.35	0.58
1:A:349:VAL:HG11	1:A:365:MET:HB2	1.89	0.54
1:A:320:THR:OG1	1:A:356:THR:OG1	2.24	0.52
1:A:358:ASP:C	1:A:358:ASP:OD1	2.48	0.52
1:A:221:ILE:HD11	2:A:443:ACT:H3	1.92	0.52
1:A:318:ASP:CG	1:A:320:THR:HG22	2.29	0.51

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:143:GLY:O	1:A:144:GLY:C	2.47	0.51
1:A:64[A]:MET:HE1	1:A:363:LEU:HB2	1.95	0.49
1:A:159:ASN:OD1	1:A:184:ASN:ND2	2.47	0.47
1:A:318:ASP:OD2	1:A:320:THR:CG2	2.50	0.47
1:A:69:ASP:HB2	1:A:71:GLY:H	1.79	0.47
1:A:252:VAL:O	1:A:274:GLY:HA2	2.17	0.43
1:A:164[A]:VAL:HG23	1:A:268:TYR:HB2	1.99	0.43
1:A:218:ARG:HD2	2:A:443:ACT:H1	2.00	0.43
1:A:420:ARG:HB2	1:A:420:ARG:CZ	2.49	0.42
1:A:164[A]:VAL:HG21	1:A:213:NFA:HD2	2.01	0.42
1:A:321:ILE:HB	1:A:357:ILE:HB	2.01	0.41
1:A:92:PHE:HB3	1:A:245:THR:HA	2.02	0.41
1:A:224:PRO:HG2	1:A:227:ILE:HD12	2.01	0.41
1:A:115:HIS:CE1	2:A:443:ACT:H2	2.48	0.41
1:A:80:LEU:HB2	1:A:291:TRP:CZ3	2.56	0.41
1:A:45:LYS:HZ1	1:A:408:GLU:CD	2.24	0.41
1:A:107:MET:HE3	1:A:108:PRO:HD2	2.03	0.40
1:A:169:SER:HA	1:A:173:HIS:O	2.20	0.40
1:A:192[A]:MET:SD	1:A:214:CRK:HE3	2.61	0.40

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 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	397/440 (90%)	382 (96%)	12 (3%)	3 (1%)	19 15

All (3) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	69	ASP
1	A	144	GLY

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Mol	Chain	Res	Type
1	A	151	SER

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	337/365 (92%)	328 (97%)	9 (3%)	44	48

All (9) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	55	ARG
1	A	89	SER
1	A	90	CYS
1	A	180	GLU
1	A	279	SER
1	A	301	GLU
1	A	335	GLN
1	A	339	GLU
1	A	408	GLU

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains ⓘ

2 non-standard protein/DNA/RNA residues 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
1	NFA	A	213	1	12,12,12	1.10	0	15,15,15	1.10	1 (6%)
1	CRK	A	214	1	24,24,24	3.25	6 (25%)	28,32,32	4.58	9 (32%)

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
1	NFA	A	213	1	-	1/8/8/8	0/1/1/1
1	CRK	A	214	1	-	1/14/31/31	0/2/2/2

All (6) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	214	CRK	CB2-CA2	11.92	1.45	1.35
1	A	214	CRK	CA2-C2	-7.21	1.41	1.48
1	A	214	CRK	CB-CA1	3.59	1.55	1.51
1	A	214	CRK	C2-N3	-3.54	1.31	1.39
1	A	214	CRK	C1-N2	3.23	1.40	1.33
1	A	214	CRK	CA2-N2	-2.75	1.32	1.38

All (10) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	214	CRK	CA2-C2-N3	17.75	111.76	103.37
1	A	214	CRK	O2-C2-CA2	-13.89	123.16	130.96
1	A	214	CRK	CD1-CE1-CZ	3.94	124.20	119.88
1	A	214	CRK	CE-SD-CG	-3.65	87.84	100.40
1	A	213	NFA	CB-CA-C	2.80	115.62	109.09
1	A	214	CRK	O3-C3-CA3	-2.42	119.10	126.39
1	A	214	CRK	CB2-CA2-C2	2.32	125.05	122.28
1	A	214	CRK	CE1-CD1-CG2	-2.22	118.35	121.25
1	A	214	CRK	CG2-CB2-CA2	2.17	132.61	129.94
1	A	214	CRK	O1-CA1-C1	2.16	123.79	121.99

There are no chirality outliers.

All (2) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	A	214	CRK	O1-CA1-CB-CG
1	A	213	NFA	O-C-CA-N

There are no ring outliers.

2 monomers are involved in 2 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
1	A	213	NFA	1	0
1	A	214	CRK	1	0

5.5 Carbohydrates [i](#)

There are no carbohydrates in this entry.

5.6 Ligand geometry [i](#)

Of 5 ligands modelled in this entry, 4 are monoatomic - leaving 1 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	ACT	A	443	-	1,3,3	2.17	1 (100%)	0,3,3	0.00	-

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	443	ACT	CH3-C	2.17	1.51	1.48

There are no bond angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

1 monomer is involved in 4 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	443	ACT	4	0

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.

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	396/440 (90%)	0.54	53 (13%) 3 4	18, 37, 88, 121	0

All (53) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	146	GLY	11.1
1	A	336	ASN	10.0
1	A	340	ALA	7.1
1	A	56	LEU	7.0
1	A	353	GLY	6.0
1	A	149	GLY	5.5
1	A	150	GLY	5.4
1	A	104	ASN	4.6
1	A	338	THR	4.5
1	A	55	ARG	4.2
1	A	347	ASN	4.2
1	A	147	GLY	3.9
1	A	70	GLY	3.9
1	A	57	SER	3.8
1	A	58	SER	3.8
1	A	144	GLY	3.5
1	A	209	LEU	3.4
1	A	314	ASP	3.3
1	A	318	ASP	3.3
1	A	324	LYS	3.3
1	A	152	MET	3.2
1	A	316	ASP	3.2
1	A	352	ASP	3.1
1	A	389	ASP	3.0
1	A	378	GLU	2.9
1	A	69	ASP	2.9
1	A	335	GLN	2.8

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Mol	Chain	Res	Type	RSRZ
1	A	357	ILE	2.7
1	A	231	PHE	2.7
1	A	317	GLY	2.7
1	A	151	SER	2.7
1	A	377	GLU	2.7
1	A	315	LYS	2.7
1	A	145	GLY	2.7
1	A	148	THR	2.7
1	A	339	GLU	2.6
1	A	320	THR	2.6
1	A	337	PRO	2.6
1	A	384	ARG	2.6
1	A	388	LYS	2.6
1	A	343	GLN	2.5
1	A	280	ASN	2.5
1	A	276[A]	ASN	2.4
1	A	206	PHE	2.4
1	A	344	ASP	2.4
1	A	380	ARG	2.3
1	A	208	ILE	2.3
1	A	210	ALA	2.3
1	A	49	ALA	2.2
1	A	349	VAL	2.2
1	A	46	THR	2.2
1	A	354	ASP	2.2
1	A	328	THR	2.1

6.2 Non-standard residues in protein, DNA, RNA chains [i](#)

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. 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	RSCC	RSR	B-factors(Å ²)	Q<0.9
1	NFA	A	213	12/12	0.90	0.21	27,28,32,33	0
1	CRK	A	214	23/23	0.96	0.15	18,19,27,33	0

6.3 Carbohydrates [i](#)

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. 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	RSCC	RSR	B-factors(Å ²)	Q<0.9
3	CA	A	446	1/1	0.72	0.09	59,59,59,59	0
2	ACT	A	443	4/4	0.89	0.18	26,32,34,42	0
3	CA	A	447	1/1	0.92	0.13	73,73,73,73	0
3	CA	A	445	1/1	0.98	0.05	33,33,33,33	0
3	CA	A	444	1/1	0.99	0.06	26,26,26,26	0

6.5 Other polymers

There are no such residues in this entry.