

Full wwPDB X-ray Structure Validation Report (i)

May 25, 2020 – 11:17 am BST

PDB ID	:	4IK8
Title	:	High resolution structure of GCaMP3 dimer form 1 at pH 7.5
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Deposited on		
$\operatorname{Resolution}$:	1.55 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 (i) symbol.

The following versions of software and data (see references (1)) were used in the production of this report:

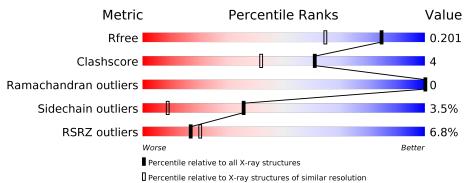
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.11
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA,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

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure: X-RAY DIFFRACTION

The reported resolution of this entry is 1.55 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	$egin{array}{c} { m Whole \ archive} \ (\#{ m Entries}) \end{array}$	${f Similar\ resolution}\ (\#{ m Entries},{ m resolution\ range}({ m \AA}))$
R_{free}	130704	1483 (1.56-1.56)
Clashscore	141614	1529 (1.56-1.56)
Ramachandran outliers	138981	1498 (1.56-1.56)
Sidechain outliers	138945	1495 (1.56-1.56)
RSRZ outliers	127900	1465 (1.56-1.56)

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 <=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	
			6%	
1	A	448	79% 8% • 12%	-

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
1	CRO	А	222	Х	-	-	-



2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 3710 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, Green fluorescent protein.

Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace		
1	А	396	Total 3163	C 1984	N 535	O 630	${ m S}$ 14	0	1	0

Chain	Residue	Modelled	Actual	Comment	Reference
А	1	MET	_	expression tag	UNP K4DIE3
A	2	GLY	-	expression tag	UNP K4DIE3
А	3	SER	_	expression tag	UNP K4DIE3
A	4	HIS	_	expression tag	UNP K4DIE3
A	5	HIS	_	expression tag	UNP K4DIE3
A	6	HIS	-	expression tag	UNP K4DIE3
A	7	HIS	-	expression tag	UNP K4DIE3
A	8	HIS	-	expression tag	UNP K4DIE3
A	9	HIS	-	expression tag	UNP K4DIE3
A	10	GLY	-	expression tag	UNP K4DIE3
A	59	LEU	-	linker	UNP K4DIE3
A	60	GLU	-	linker	UNP K4DIE3
A	65	LYS	MET	engineered mutation	UNP P42212
A	75	ALA	VAL	engineered mutation	UNP P42212
A	87	GLY	SER	engineered mutation	UNP P42212
A	92	TYR	ASP	engineered mutation	UNP P42212
A	115	VAL	THR	engineered mutation	UNP P42212
A	118	LYS	ALA	engineered mutation	UNP P42212
A	143	LEU	HIS	engineered mutation	UNP P42212
A	151	GLY	-	linker	UNP P42212
А	152	GLY	-	linker	UNP P42212
А	153	THR	-	linker	UNP P42212
А	154	GLY	-	linker	UNP P42212
А	155	GLY	-	linker	UNP P42212
А	156	SER	-	linker	UNP P42212
А	157	MET	-	linker	UNP P42212
А	158	VAL	-	linker	UNP P42212

There are 33 discrepancies between the modelled and reference sequences:

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Chain	Residue	Modelled	Actual	Comment	Reference
А	221	LEU	PHE	engineered mutation	UNP P42212
A	222	CRO	SER	$\operatorname{chromophore}$	UNP P42212
А	222	CRO	TYR	chromophore	UNP P42212
A	222	CRO	GLY	$\operatorname{chromophore}$	UNP P42212
А	250	ILE	VAL	engineered mutation	UNP P42212
А	372	THR	ILE	engineered mutation	UNP K4DIE3

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• Molecule 2 is CALCIUM ION (three-letter code: CA) (formula: Ca).

M	[o]	Chain	Residues	Atoms	ZeroOcc	AltConf
	2	А	4	Total Ca 4 4	0	0

• Molecule 3 is water.

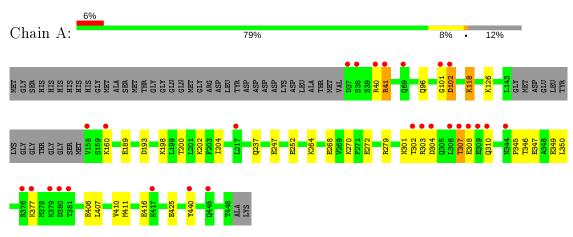
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	А	543	Total O 543 543	0	0



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 (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, Green fluorescent protein





4 Data and refinement statistics (i)

Property	Value	Source
Space group	C 1 2 1	Depositor
Cell constants	126.34Å 47.35 Å 66.74 Å	Depositor
a, b, c, α , β , γ	90.00° 100.27° 90.00°	Depositor
Resolution (Å)	25.60 - 1.55	Depositor
Resolution (A)	25.60 - 1.48	EDS
% Data completeness	98.3 (25.60-1.55)	Depositor
(in resolution range)	98.3 (25.60-1.48)	EDS
R _{merge}	(Not available)	Depositor
R _{sym}	(Not available)	Depositor
$< I/\sigma(I) > 1$	$2.66 (at 1.48 \text{\AA})$	Xtriage
Refinement program	PHENIX (phenix.refine: 1.7.3_928)	Depositor
D D.	0.179 , 0.209	Depositor
R, R_{free}	0.173 , 0.201	DCC
R_{free} test set	3246 reflections $(5.07%)$	wwPDB-VP
Wilson B-factor $(Å^2)$	21.2	Xtriage
Anisotropy	0.060	Xtriage
Bulk solvent $k_{sol}(e/Å^3), B_{sol}(Å^2)$	0.38 , 51.6	EDS
L-test for twinning ²	$< L >=0.50, < L^2>=0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.97	EDS
Total number of atoms	3710	wwPDB-VP
Average B, all atoms $(Å^2)$	28.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 7.25% of the height of the origin peak. No significant pseudotranslation is detected.

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.



¹Intensities estimated from amplitudes.

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: CA, CRO

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		
	Unam	RMSZ	# Z > 5	RMSZ	# Z > 5	
1	А	0.57	0/3199	0.70	0/4307	

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	А	1	0

There are no bond length outliers.

There are no bond angle outliers.

All (1) chirality outliers are listed below:

Mol	Chain	Res	Type	Atom
1	А	222	CRO	CA1

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	3163	0	3062	$\overline{28}$	2

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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 4.

All (28) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic	Clash
Atom-1	Atom-2	${ m distance}~({ m \AA})$	overlap (Å)
1:A:349:GLU:OE1	3:A:753:HOH:O	1.91	0.87
1:A:268:GLU:OE1	3:A:791:HOH:O	1.96	0.83
1:A:347:GLU:OE1	3:A:783:HOH:O	1.97	0.82
1:A:252:GLU:OE2	3:A:1123:HOH:O	2.01	0.79
1:A:102:ASP:OD1	1:A:102:ASP:N	2.15	0.69
1:A:440:TYR:OH	3:A:889:HOH:O	2.12	0.68
1:A:237:GLN:NE2	3:A:1083:HOH:O	2.06	0.68
1:A:189:GLU:OE1	3:A:1077:HOH:O	2.12	0.67
1:A:268:GLU:OE1	1:A:270:LYS:NZ	2.28	0.65
1:A:41:ARG:NH1	3:A:948:HOH:O	2.07	0.64
1:A:301:ASN:OD1	3:A:956:HOH:O	2.16	0.61
1:A:346:THR:HB	3:A:1020:HOH:O	2.04	0.58
1:A:425:GLU:OE1	3:A:927:HOH:O	2.17	0.57
1:A:272:GLU:OE2	1:A:279:ARG:NH2	2.25	0.57
1:A:307:THR:HG22	1:A:310:GLN:H	1.68	0.56
1:A:101:GLY:HA3	1:A:247:GLU:OE2	2.06	0.55
1:A:41:ARG:HB2	1:A:41:ARG:CZ	2.38	0.54
1:A:247:GLU:OE1	3:A:807:HOH:O	2.19	0.51
1:A:202:LYS:HE2	1:A:204:ILE:HD11	1.93	0.51
1:A:118:LYS:HE3	1:A:118:LYS:HA	1.98	0.45
1:A:247:GLU:CD	3:A:807:HOH:O	2.54	0.45
1:A:264:LYS:HB3	1:A:264:LYS:HE3	1.75	0.43
1:A:406:GLU:O	1:A:410:VAL:HG23	2.18	0.43
1:A:198:LYS:HE2	1:A:200:THR:HG23	2.02	0.41
1:A:407:LEU:O	1:A:411:MET:HG2	2.20	0.41
1:A:247:GLU:HG3	3:A:785:HOH:O	2.19	0.41
1:A:345:PRO:HB2	1:A:350:LEU:HG	2.02	0.41
1:A:193:ASP:OD1	3:A:656:HOH:O	2.21	0.40

All (4) symmetry-related close contacts are listed below. The label for Atom-2 includes the symmetry operator and encoded unit-cell translations to be applied.



Chain Non-H Mol H(model) H(added) Clashes Symm-Clashes 2 А 0 0 4 0 0 3 А 543 0 0 154 All All 37100 3062284

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:303:ARG:NH2	3:A:853:HOH:O[1_565]	1.86	0.34
3:A:1006:HOH:O	3:A:1062:HOH:O[2_655]	1.87	0.33
1:A:96:GLN:NE2	3:A:898:HOH:O[3_445]	1.95	0.25
3:A:811:HOH:O	3:A:879:HOH:O[3_445]	2.10	0.10

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	А	390/448~(87%)	386~(99%)	4 (1%)	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 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	А	343/381~(90%)	331~(96%)	12~(4%)	36 9		

All (12) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	А	40	ARG
1	А	41	ARG
1	А	102	ASP
1	А	118	LYS
1	А	126	LYS

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Mol	Chain	\mathbf{Res}	Type
1	А	160	LYS
1	А	302	THR
1	А	304	ASP
1	А	307	THR
1	А	308	GLU
1	А	377	LYS
1	А	416	GLU

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	\mathbf{Res}	Type
1	А	413	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)

1 non-standard protein/DNA/RNA residue 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	\mathbf{ths}	В	ond ang	gles
Noi Type Chai	Ullalli	Res Lillk		Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z >2	
1	CRO	А	222	1	23, 23, 24	2.29	6 (26%)	30,32,34	2.48	10 (33%)

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	\mathbf{Res}	Link	Chirals	Torsions	Rings
1	CRO	А	222	1	1/1/6/8	3/12/31/32	0/2/2/2



Mol	Chain	Res	Type	Atoms	Z	$\operatorname{Observed}(\operatorname{\AA})$	Ideal(Å)
1	А	222	CRO	CA1-C1	-5.60	1.43	1.51
1	А	222	CRO	C1-N3	4.72	1.45	1.37
1	А	222	CRO	CA2-C2	-4.67	1.44	1.48
1	А	222	CRO	CB2-CA2	3.75	1.38	1.35
1	А	222	CRO	CA3-N3	-3.06	1.41	1.47
1	А	222	CRO	C2-N3	2.57	1.45	1.39

All (6) bond length outliers are listed below:

All (10) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
1	А	222	CRO	OG1-CB1-CA1	-7.22	93.59	109.04
1	А	222	CRO	CA2-N2-C1	5.82	110.06	105.77
1	А	222	CRO	CA1-C1-N3	4.90	130.62	124.75
1	А	222	CRO	CA1-C1-N2	-3.66	118.76	123.89
1	А	222	CRO	C2-N3-C1	-3.41	106.24	107.97
1	А	222	CRO	O3-C3-CA3	-3.08	117.10	126.39
1	А	222	CRO	CA2-C2-N3	3.07	104.82	103.37
1	А	222	CRO	CB2-CA2-C2	2.35	125.08	122.28
1	А	222	CRO	N3-C1-N2	-2.08	110.02	111.45
1	А	222	CRO	CB2-CA2-N2	-2.01	126.04	128.83

All (1) chirality outliers are listed below:

Mol	Chain	Res	Type	Atom
1	А	222	CRO	CA1

All (3) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	А	222	CRO	N1-CA1-CB1-CG1
1	А	222	CRO	C2-CA2-CB2-CG2
1	А	222	CRO	N2-CA2-CB2-CG2

There are no ring outliers.

No monomer is involved in short contacts.

5.5 Carbohydrates (i)

There are no carbohydrates in this entry.



5.6 Ligand geometry (i)

Of 4 ligands modelled in this entry, 4 are monoatomic - leaving 0 for Mogul analysis. There are no bond length outliers. There are no bond angle outliers. There are no chirality outliers. There are no torsion outliers. There are no ring outliers. No monomer is involved in short contacts.

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 (i)

6.1 Protein, DNA and RNA chains (i)

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	А	395/448~(88%)	0.32	27 (6%) 17 20	12, 24, 46, 68	0

All (27) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	А	158	VAL	8.5
1	А	304	ASP	7.1
1	А	377	LYS	4.9
1	А	37	ASP	4.8
1	А	379	LYS	4.0
1	А	417	LYS	3.7
1	А	380	ASP	3.6
1	А	40	ARG	3.3
1	А	38	SER	3.2
1	А	303	ARG	3.1
1	А	309	GLU	3.0
1	А	306	LEU	3.0
1	А	160	LYS	2.9
1	А	41	ARG	2.7
1	А	445	GLN	2.7
1	А	310	GLN	2.6
1	А	376	ARG	2.5
1	А	307	THR	2.4
1	А	344	ASN	2.3
1	А	302	THR	2.3
1	А	102	ASP	2.3
1	А	69	GLN	2.3
1	А	440	TYR	2.2
1	А	101	GLY	2.2
1	А	381	THR	2.2
1	А	217	LEU	2.1
1	А	308	GLU	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, 95^{th} 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	$\mathbf{B} ext{-factors}(\mathbf{\AA}^2)$	$Q{<}0.9$
1	CRO	А	222	22/23	0.96	0.10	$12,\!15,\!19,\!20$	0

6.3 Carbohydrates (i)

There are no carbohydrates in this entry.

6.4 Ligands (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, 95^{th} 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	\mathbf{RSR}	$\mathbf{B} ext{-factors}(\mathbf{\mathring{A}}^2)$	$Q{<}0.9$
2	CA	А	503	1/1	0.94	0.09	$25,\!25,\!25,\!25$	0
2	CA	А	504	1/1	0.96	0.08	$26,\!26,\!26,\!26$	0
2	CA	А	501	1/1	0.98	0.04	22,22,22,22	0
2	CA	А	502	1/1	0.99	0.04	$18,\!18,\!18,\!18$	0

6.5 Other polymers (i)

There are no such residues in this entry.

