Full wwPDB X-ray Structure Validation Report (i)

Aug 22, 2023 – 11:06 PM EDT

PDB ID	:	3EKJ
Title	:	Calcium-free GCaMP2 (calcium binding deficient mutant)
Authors	:	Akerboom, J.; Velez Rivera, J.D.; Looger, L.L.; Schreiter, E.R.
Deposited on	:	2008-09-19
Resolution	:	2.80 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 types of validation reports are described at
http://www.wwpdb.org/validation/2017/FAQs#types.

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

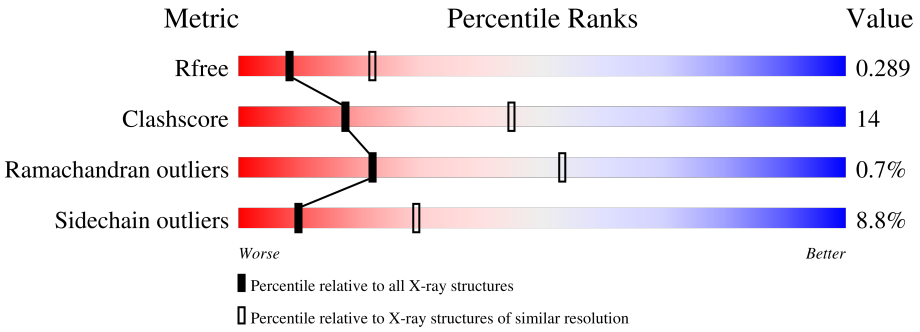
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.35
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.35

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 2.80 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	3140 (2.80-2.80)
Clashscore	141614	3569 (2.80-2.80)
Ramachandran outliers	138981	3498 (2.80-2.80)
Sidechain outliers	138945	3500 (2.80-2.80)

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 of 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%

Mol	Chain	Length	Quality of chain
1	A	449	47% 18% • 33%

2 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 2405 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 Myosin light chain kinase, Green fluorescent protein, Calmodulin chimera.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	302	2400	1522	400	466	12	0	0	0

There are 28 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	76	ALA	VAL	see remark 999	UNP P42212
A	88	GLY	SER	see remark 999	UNP P42212
A	93	TYR	ASP	see remark 999	UNP P42212
A	119	LYS	ALA	see remark 999	UNP P42212
A	144	LEU	HIS	see remark 999	UNP P42212
A	152	GLY	-	linker	UNP P42212
A	153	GLY	-	linker	UNP P42212
A	154	THR	-	linker	UNP P42212
A	155	GLY	-	linker	UNP P42212
A	156	GLY	-	linker	UNP P42212
A	157	SER	-	linker	UNP P42212
A	158	MET	-	linker	UNP P42212
A	159	VAL	-	linker	UNP P42212
A	222	LEU	PHE	see remark 999	UNP P42212
A	224	CRO	SER	chromophore	UNP P42212
A	?	-	TYR	chromophore	UNP P42212
A	?	-	GLY	chromophore	UNP P42212
A	251	ILE	VAL	see remark 999	UNP P42212
A	303	THR	-	linker	UNP P42212
A	304	ARG	-	linker	UNP P42212
A	329	GLY	THR	engineered mutation	UNP P0DP29
A	334	GLN	GLU	engineered mutation	UNP P0DP29
A	359	GLY	ASP	engineered mutation	UNP P0DP29
A	370	GLN	GLU	engineered mutation	UNP P0DP29
A	396	GLY	ASP	engineered mutation	UNP P0DP29
A	407	GLN	GLU	engineered mutation	UNP P0DP29

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Chain	Residue	Modelled	Actual	Comment	Reference
A	432	GLY	ASP	engineered mutation	UNP P0DP29
A	443	GLN	GLU	engineered mutation	UNP P0DP29

- Molecule 2 is water.

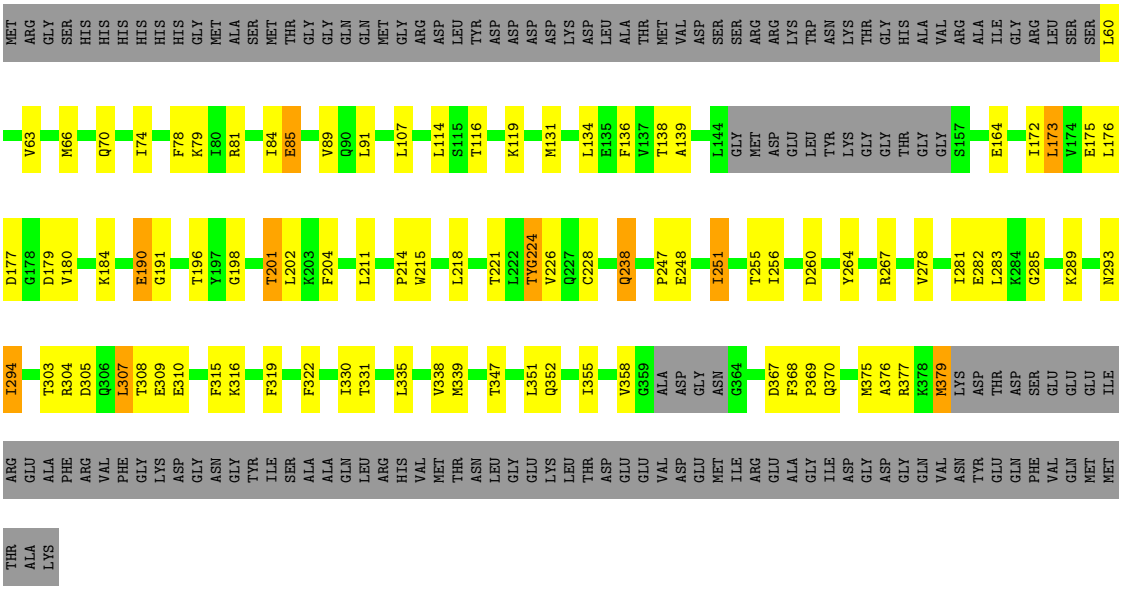
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
2	A	5	5	5	0	0

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: Myosin light chain kinase, Green fluorescent protein, Calmodulin chimera

Chain A: 47% 18% • 33%



4 Data and refinement statistics (i)

Property	Value	Source
Space group	C 1 2 1	Depositor
Cell constants a, b, c, α, β, γ	211.87Å 47.67Å 42.99Å 90.00° 97.61° 90.00°	Depositor
Resolution (Å)	31.88 – 2.80 31.88 – 2.12	Depositor EDS
% Data completeness (in resolution range)	98.4 (31.88-2.80) 93.9 (31.88-2.12)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	0.15	Depositor
$< I/\sigma(I) >$ [1]	1.02 (at 2.12Å)	Xtriage
Refinement program	REFMAC 5.2.0019	Depositor
R, R_{free}	0.209 , 0.280 0.214 , 0.289	Depositor DCC
R_{free} test set	1159 reflections (5.06%)	wwPDB-VP
Wilson B-factor (Å^2)	26.5	Xtriage
Anisotropy	0.664	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.37 , 17.1	EDS
L-test for twinning[2]	$< \|L\| > = 0.47$, $< L^2 > = 0.30$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.91	EDS
Total number of atoms	2405	wwPDB-VP
Average B, all atoms (Å^2)	30.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 8.76% of the height of the origin peak. No significant pseudotranslation is detected.*

[1] Intensities estimated from amplitudes.

[2] Theoretical values of $< |L| >$, $< L^2 >$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

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: 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	
		RMSZ	$\#\|Z\| >5$	RMSZ	$\#\|Z\| >5$
1	A	0.74	0/2422	0.76	0/3261

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	A	2400	0	2351	65	0
2	A	5	0	0	0	0
All	All	2405	0	2351	65	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.

All (65) 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:138:THR:HG23	1:A:367:ASP:OD2	1.84	0.77
1:A:134:LEU:CD1	1:A:201:THR:HG23	2.16	0.76

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:367:ASP:HB3	1:A:369:PRO:HD2	1.66	0.76
1:A:138:THR:CG2	1:A:367:ASP:OD2	2.37	0.72
1:A:260:ASP:O	1:A:289:LYS:HE2	1.91	0.71
1:A:134:LEU:HD13	1:A:201:THR:CG2	2.21	0.70
1:A:375:MET:O	1:A:379:MET:HE2	1.94	0.68
1:A:375:MET:O	1:A:379:MET:CE	2.43	0.67
1:A:176:LEU:C	1:A:176:LEU:HD23	2.18	0.63
1:A:173:LEU:HD23	1:A:278:VAL:HG22	1.80	0.63
1:A:134:LEU:HD13	1:A:201:THR:HG23	1.78	0.63
1:A:294:ILE:N	1:A:294:ILE:HD12	2.15	0.61
1:A:89:VAL:O	1:A:89:VAL:HG12	2.02	0.59
1:A:81:ARG:NH1	1:A:89:VAL:HG11	2.17	0.59
1:A:247:PRO:HD2	1:A:248:GLU:OE1	2.01	0.59
1:A:251:ILE:HD12	1:A:267:ARG:NH2	2.19	0.58
1:A:221:THR:HG22	1:A:281:ILE:HD13	1.86	0.57
1:A:322:PHE:CD1	1:A:338:VAL:HG22	2.41	0.56
1:A:107:LEU:HD13	1:A:238:GLN:O	2.06	0.55
1:A:307:LEU:HD11	1:A:376:ALA:HA	1.88	0.55
1:A:335:LEU:HD23	1:A:351:LEU:HD23	1.89	0.55
1:A:79:LYS:HD2	1:A:91:LEU:HD22	1.88	0.54
1:A:294:ILE:N	1:A:294:ILE:CD1	2.71	0.54
1:A:335:LEU:O	1:A:339:MET:HG2	2.07	0.54
1:A:176:LEU:HD23	1:A:177:ASP:N	2.23	0.53
1:A:358:VAL:HG11	1:A:370:GLN:HB3	1.90	0.52
1:A:211:LEU:HD22	1:A:215:TRP:CE2	2.45	0.52
1:A:131:MET:HE3	1:A:204:PHE:CD1	2.45	0.51
1:A:319:PHE:CZ	1:A:330:ILE:HG23	2.46	0.51
1:A:358:VAL:HG23	1:A:377:ARG:NH1	2.24	0.51
1:A:164:GLU:HA	1:A:164:GLU:OE1	2.10	0.50
1:A:63:VAL:HG13	1:A:78:PHE:CD2	2.46	0.50
1:A:84:ILE:HD13	1:A:293:ASN:HD21	1.76	0.50
1:A:375:MET:O	1:A:379:MET:HE1	2.13	0.48
1:A:293:ASN:C	1:A:294:ILE:HD12	2.33	0.48
1:A:138:THR:HG21	1:A:367:ASP:OD2	2.13	0.48
1:A:214:PRO:HD2	1:A:294:ILE:HG23	1.96	0.47
1:A:74:ILE:C	1:A:74:ILE:HD12	2.34	0.47
1:A:180:VAL:HG22	1:A:285:GLY:HA3	1.97	0.47
1:A:224:CRO:N2	1:A:224:CRO:HD1	2.31	0.46
1:A:81:ARG:HH11	1:A:89:VAL:HG11	1.81	0.46
1:A:172:ILE:HB	1:A:202:LEU:HD21	1.97	0.46
1:A:283:LEU:C	1:A:283:LEU:HD23	2.36	0.46

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:256:ILE:HD12	1:A:264:TYR:HB2	1.99	0.45
1:A:352:GLN:HA	1:A:355:ILE:HD12	1.98	0.45
1:A:221:THR:CG2	1:A:281:ILE:HG21	2.47	0.44
1:A:85:GLU:HA	1:A:85:GLU:OE1	2.18	0.44
1:A:358:VAL:HG23	1:A:377:ARG:HH12	1.81	0.44
1:A:248:GLU:H	1:A:248:GLU:CD	2.21	0.43
1:A:191:GLY:N	1:A:202:LEU:HD23	2.34	0.43
1:A:60:LEU:HD23	1:A:60:LEU:HA	1.94	0.42
1:A:190:GLU:C	1:A:202:LEU:HD23	2.40	0.42
1:A:191:GLY:HA3	1:A:202:LEU:HD23	2.02	0.41
1:A:211:LEU:HD21	1:A:218:LEU:HD12	2.02	0.41
1:A:179:ASP:HB2	1:A:184:LYS:HZ2	1.85	0.41
1:A:134:LEU:HD13	1:A:201:THR:HG22	2.03	0.41
1:A:116:THR:HG21	1:A:224:CRO:CZ	2.51	0.41
1:A:136:PHE:HA	1:A:198:GLY:O	2.20	0.41
1:A:251:ILE:HD12	1:A:267:ARG:CZ	2.50	0.41
1:A:316:LYS:HA	1:A:368:PHE:CE1	2.56	0.41
1:A:107:LEU:HD13	1:A:238:GLN:C	2.42	0.40
1:A:226:VAL:O	1:A:226:VAL:HG23	2.21	0.40
1:A:315:PHE:CZ	1:A:375:MET:HE2	2.57	0.40
1:A:114:LEU:HD23	1:A:139:ALA:HA	2.03	0.40
1:A:138:THR:OG1	1:A:369:PRO:HB2	2.21	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	293/449 (65%)	273 (93%)	18 (6%)	2 (1%)	22 53

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	85	GLU
1	A	304	ARG

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	A	261/378 (69%)	238 (91%)	23 (9%)	10 29

All (23) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	66	MET
1	A	70	GLN
1	A	119	LYS
1	A	173	LEU
1	A	175	GLU
1	A	190	GLU
1	A	196	THR
1	A	201	THR
1	A	228	CYS
1	A	238	GLN
1	A	251	ILE
1	A	255	THR
1	A	282	GLU
1	A	294	ILE
1	A	303	THR
1	A	305	ASP
1	A	307	LEU
1	A	308	THR
1	A	309	GLU
1	A	310	GLU
1	A	331	THR
1	A	347	THR
1	A	379	MET

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are

no such sidechains identified.

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	Bond lengths			Bond angles		
					Counts	RMSZ	$\#\|Z\| > 2$	Counts	RMSZ	$\#\|Z\| > 2$
1	CRO	A	224	1	23,23,24	3.28	6 (26%)	30,32,34	3.48	8 (26%)

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	CRO	A	224	1	-	0/12/31/32	0/2/2/2

All (6) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	224	CRO	CB2-CA2	13.48	1.46	1.35
1	A	224	CRO	CA2-C2	-4.48	1.44	1.48
1	A	224	CRO	C1-N2	4.01	1.38	1.32
1	A	224	CRO	C2-N3	-3.78	1.31	1.39
1	A	224	CRO	O2-C2	2.42	1.28	1.23
1	A	224	CRO	CA1-C1	-2.24	1.48	1.51

All (8) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	224	CRO	CA2-C2-N3	12.77	109.41	103.37
1	A	224	CRO	O2-C2-CA2	-10.00	125.35	130.96
1	A	224	CRO	C2-N3-C1	-4.60	105.64	107.97
1	A	224	CRO	C1-CA1-N1	-4.12	103.28	109.96
1	A	224	CRO	CA1-C1-N3	-3.75	120.25	124.75
1	A	224	CRO	CG2-CB2-CA2	-3.66	125.46	129.94
1	A	224	CRO	CA3-N3-C1	2.77	130.49	127.16
1	A	224	CRO	CA1-C1-N2	2.11	126.84	123.89

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

1 monomer is involved in 2 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
1	A	224	CRO	2	0

5.5 Carbohydrates (i)

There are no monosaccharides in this entry.

5.6 Ligand geometry (i)

There are no ligands in this entry.

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)

Unable to reproduce the depositors R factor - this section is therefore empty.

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

Unable to reproduce the depositors R factor - this section is therefore empty.

6.3 Carbohydrates (i)

Unable to reproduce the depositors R factor - this section is therefore empty.

6.4 Ligands (i)

Unable to reproduce the depositors R factor - this section is therefore empty.

6.5 Other polymers (i)

Unable to reproduce the depositors R factor - this section is therefore empty.