

wwPDB X-ray Structure Validation Summary Report (i)

Nov 26, 2024 – 02:14 PM JST

PDB ID : 8JLU

 $\label{thm:continuous} \mbox{Title} \quad : \quad \mbox{Crystal structure of the Green fluorescent protein SET203EF223DA227 variant}$

at pH 8.5

Authors : Shin, S.C. Deposited on : 2023-06-02

Resolution : 2.09 Å(reported)

This is a wwPDB X-ray Structure Validation Summary 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 (1)) were used in the production of this report:

Mol Probity : 4.02b-467

Mogul: 1.8.5 (274361), CSD as541be (2020)

Xtriage (Phenix) : 1.21 EDS : 3.0

Percentile statistics : 20231227.v01 (using entries in the PDB archive December 27th 2023)

CCP4 : 9.0.004 (Gargrove)

Density-Fitness : 1.0.11

Ideal geometry (proteins) : Engh & Huber (2001) Ideal geometry (DNA, RNA) : Parkinson et al. (1996)

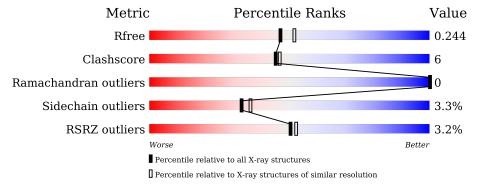
Validation Pipeline (wwPDB-VP) : 2.40

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 2.09 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	$\begin{array}{c} \textbf{Whole archive} \\ (\#\text{Entries}) \end{array}$	$\begin{array}{c} {\rm Similar\ resolution} \\ (\#{\rm Entries},\ {\rm resolution\ range}({\rm \AA})) \end{array}$
R_{free}	164625	6234 (2.10-2.10)
Clashscore	180529	6893 (2.10-2.10)
Ramachandran outliers	177936	6839 (2.10-2.10)
Sidechain outliers	177891	6840 (2.10-2.10)
RSRZ outliers	164620	6234 (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 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% 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	236	82%	13%	5%
1	В	236	83%	10%	• 5%
1	С	236	81%	14%	• 5%
1	D	236	82%	11%	• 5%



2 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 7589 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 Green fluorescent protein.

Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	Trace	
1	Λ	225	Total	С	N	О	S	0	0	0
1	A	229	1801	1146	302	347	6	0	0	
1	В	225	Total	С	N	О	S	0	0	0
1	Ъ	229	1801	1146	302	347	6	0	0	
1	С	225	Total	С	N	О	S	0	0	0
1		220	1801	1146	302	347	6	0	0	
1	1 D	225	Total	С	N	О	S	0	0	0
1		225	1801	1146	302	347	6	0	U	

There are 52 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	64	LEU	PHE	engineered mutation	UNP P42212
A	66	CRO	SER	chromophore	UNP P42212
A	66	CRO	TYR	chromophore	UNP P42212
A	66	CRO	GLY	chromophore	UNP P42212
A	80	ARG	GLN	engineered mutation	UNP P42212
A	147	ASP	SER	engineered mutation	UNP P42212
A	149	GLN	ASN	engineered mutation	UNP P42212
A	163	ALA	VAL	engineered mutation	UNP P42212
A	175	GLY	SER	engineered mutation	UNP P42212
A	202	PHE	SER	engineered mutation	UNP P42212
A	204	GLU	GLN	engineered mutation	UNP P42212
A	206	THR	ALA	engineered mutation	UNP P42212
A	223	ASP	PHE	engineered mutation	UNP P42212
В	64	LEU	PHE	engineered mutation	UNP P42212
В	66	CRO	SER	chromophore	UNP P42212
В	66	CRO	TYR	chromophore	UNP P42212
В	66	CRO	GLY	chromophore	UNP P42212
В	80	ARG	GLN	engineered mutation	UNP P42212
В	147	ASP	SER	engineered mutation	UNP P42212
В	149	GLN	ASN	engineered mutation	UNP P42212
В	163	ALA	VAL	engineered mutation	UNP P42212

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Chain	Residue	Modelled	Actual	Comment	Reference
В	175	GLY	SER	engineered mutation	UNP P42212
В	202	PHE	SER	engineered mutation	UNP P42212
В	204	GLU	GLN	engineered mutation	UNP P42212
В	206	THR	ALA	engineered mutation	UNP P42212
В	223	ASP	PHE	engineered mutation	UNP P42212
С	64	LEU	PHE	engineered mutation	UNP P42212
С	66	CRO	SER	chromophore	UNP P42212
С	66	CRO	TYR	chromophore	UNP P42212
С	66	CRO	GLY	chromophore	UNP P42212
С	80	ARG	GLN	engineered mutation	UNP P42212
С	147	ASP	SER	engineered mutation	UNP P42212
С	149	GLN	ASN	engineered mutation	UNP P42212
С	163	ALA	VAL	engineered mutation	UNP P42212
С	175	GLY	SER	engineered mutation	UNP P42212
С	202	PHE	SER	engineered mutation	UNP P42212
С	204	GLU	GLN	engineered mutation	UNP P42212
С	206	THR	ALA	engineered mutation	UNP P42212
С	223	ASP	PHE	engineered mutation	UNP P42212
D	64	LEU	PHE	engineered mutation	UNP P42212
D	66	CRO	SER	chromophore	UNP P42212
D	66	CRO	TYR	chromophore	UNP P42212
D	66	CRO	GLY	chromophore	UNP P42212
D	80	ARG	GLN	engineered mutation	UNP P42212
D	147	ASP	SER	engineered mutation	UNP P42212
D	149	GLN	ASN	engineered mutation	UNP P42212
D	163	ALA	VAL	engineered mutation	UNP P42212
D	175	GLY	SER	engineered mutation	UNP P42212
D	202	PHE	SER	engineered mutation	UNP P42212
D	204	GLU	GLN	engineered mutation	UNP P42212
D	206	THR	ALA	engineered mutation	UNP P42212
D	223	ASP	PHE	engineered mutation	UNP P42212

• Molecule 2 is water.

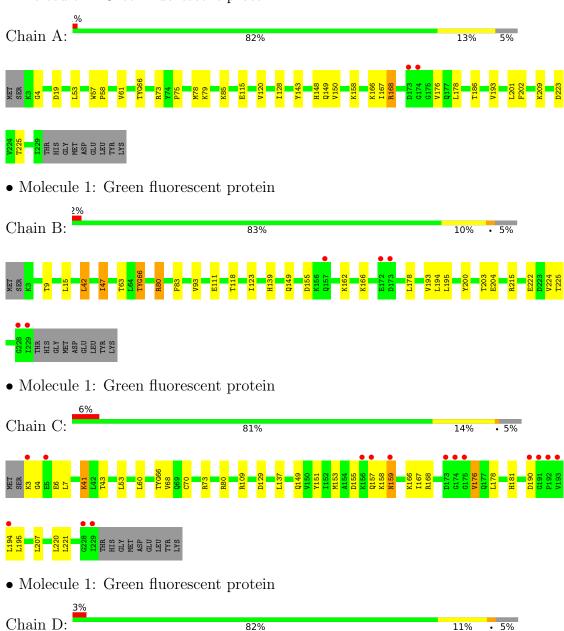
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
2	A	117	Total O 117 117	0	0
2	В	84	Total O 84 84	0	0
2	С	104	Total O 104 104	0	0
2	D	80	Total O 80 80	0	0



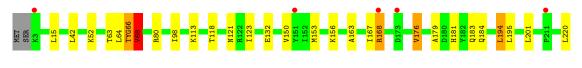
3 Residue-property plots (i)

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 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: Green fluorescent protein











4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants	70.83Å 46.30Å 146.59Å	Donositor
a, b, c, α , β , γ	90.00° 93.78° 90.00°	Depositor
Resolution (Å)	49.23 - 2.09	Depositor
Resolution (A)	49.23 - 2.09	EDS
% Data completeness	83.5 (49.23-2.09)	Depositor
(in resolution range)	83.5 (49.23-2.09)	EDS
R_{merge}	0.08	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) > 1$	2.88 (at 2.10Å)	Xtriage
Refinement program	PHENIX (1.19.2_4158: ???)	Depositor
P. P.	0.193 , 0.245	Depositor
R, R_{free}	0.194 , 0.244	DCC
R_{free} test set	54599 reflections $(4.23%)$	wwPDB-VP
Wilson B-factor (Å ²)	21.7	Xtriage
Anisotropy	0.385	Xtriage
Bulk solvent $k_{sol}(e/Å^3)$, $B_{sol}(Å^2)$	$0.35 \; , \; 35.5$	EDS
L-test for twinning ²	$ < L > = 0.48, < L^2> = 0.31$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.93	EDS
Total number of atoms	7589	wwPDB-VP
Average B, all atoms (Å ²)	26.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 5.21% of the height of the origin peak. No significant pseudotranslation is detected.

²Theoretical 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.



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: 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		
IVIOI	Chain	RMSZ $ \# Z > 5$		RMSZ	# Z > 5	
1	A	0.41	0/1819	0.65	0/2459	
1	В	0.41	0/1819	0.63	1/2459 (0.0%)	
1	С	0.50	0/1819	0.81	3/2459 (0.1%)	
1	D	0.39	0/1819	0.68	3/2459 (0.1%)	
All	All	0.43	0/7276	0.70	7/9836 (0.1%)	

There are no bond length outliers.

The worst 5 of 7 bond angle outliers are listed below:

Mol	Chain	Res	Type	${f Atoms}$	\mathbf{Z}	$\mathbf{Observed}(^{o})$	$\operatorname{Ideal}({}^{o})$
1	С	195	LEU	N-CA-CB	-18.68	73.04	110.40
1	D	64	LEU	CA-C-O	-13.42	91.92	120.10
1	С	195	LEU	N-CA-C	8.91	135.06	111.00
1	D	68	VAL	N-CA-C	-6.09	94.57	111.00
1	В	42	LEU	CA-CB-CG	5.26	127.41	115.30

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	1801	0	1723	16	0
1	В	1801	0	1723	22	0

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Continued	trom	mmoninonic	maaa
COHABABACA		DIEUIUU	DUIUE
0 0 1000100000			

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	С	1801	0	1723	22	0
1	D	1801	0	1722	23	0
2	A	117	0	0	5	0
2	В	84	0	0	1	0
2	С	104	0	0	6	0
2	D	80	0	0	3	0
All	All	7589	0	6891	82	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.

The worst 5 of 82 close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	$\begin{array}{c} {\rm Interatomic} \\ {\rm distance} \ ({\rm \AA}) \end{array}$	Clash overlap (Å)
1:B:66:CRO:CE1	1:B:203:THR:HG21	1.91	1.01
1:B:15:LEU:HD23	1:B:118:THR:HG21	1.68	0.75
1:D:168:ARG:NE	1:D:176:VAL:HG21	2.02	0.74
1:C:41:LYS:CE	2:C:354:HOH:O	2.37	0.73
1:D:168:ARG:NE	1:D:176:VAL:CG2	2.55	0.70

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	Perce	entiles
1	A	220/236~(93%)	215 (98%)	5 (2%)	0	100	100
1	В	220/236 (93%)	217 (99%)	3 (1%)	0	100	100
1	С	220/236 (93%)	213 (97%)	7 (3%)	0	100	100
1	D	220/236 (93%)	214 (97%)	6 (3%)	0	100	100
All	All	880/944 (93%)	859 (98%)	21 (2%)	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	Rotameric Outliers	
1	A	191/205~(93%)	185 (97%)	6 (3%)	35 39
1	В	191/205~(93%)	187 (98%)	4 (2%)	48 55
1	С	191/205~(93%)	183 (96%)	8 (4%)	25 26
1	D	191/205 (93%)	184 (96%)	7 (4%)	29 31
All	All	$764/820 \ (93\%)$	739 (97%)	25 (3%)	33 36

5 of 25 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	С	129	ASP
1	С	194	LEU
1	D	225	THR
1	С	176	VAL
1	D	52	LYS

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

Mol	Chain	Res	Type
1	A	184	GLN
1	С	159	ASN
1	D	164	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)

4 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).

Mal	Mol Type	Chain	Res	Link	Bond lengths			Bond angles		
IVIOI	туре	Chain	nes		Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z > 2
1	CRO	В	66	1	23,23,24	6.06	10 (43%)	30,32,34	3.33	10 (33%)
1	CRO	D	66	1	23,23,24	6.05	10 (43%)	30,32,34	4.63	11 (36%)
1	CRO	С	66	1	23,23,24	5.93	11 (47%)	30,32,34	3.89	14 (46%)
1	CRO	A	66	1	23,23,24	6.07	10 (43%)	30,32,34	3.33	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	Res	Link	Chirals	Torsions	Rings
1	CRO	В	66	1	-	1/12/31/32	0/2/2/2
1	CRO	D	66	1	-	5/12/31/32	0/2/2/2
1	CRO	С	66	1	-	3/12/31/32	0/2/2/2
1	CRO	A	66	1	-	1/12/31/32	0/2/2/2

The worst 5 of 41 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$\operatorname{Observed}(\text{\AA})$	Ideal(Å)
1	A	66	CRO	CB2-CA2	20.84	1.52	1.35
1	В	66	CRO	CB2-CA2	20.80	1.52	1.35
1	D	66	CRO	CB2-CA2	20.61	1.52	1.35
1	С	66	CRO	CB2-CA2	19.13	1.51	1.35
1	A	66	CRO	CA2-C2	-12.88	1.35	1.48

The worst 5 of 45 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$\mathbf{Observed}(^o)$	$Ideal(^{o})$
1	D	66	CRO	C1-CA1-N1	15.86	135.71	109.96
1	D	66	CRO	CA2-C2-N3	10.68	108.42	103.37
1	В	66	CRO	CA2-C2-N3	9.28	107.76	103.37
1	A	66	CRO	CA2-C2-N3	9.25	107.75	103.37
1	С	66	CRO	CA2-C2-N3	9.16	107.70	103.37



There are no chirality outliers.

5 of 10 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	С	66	CRO	C1-CA1-CB1-CG1
1	С	66	CRO	C1-CA1-CB1-OG1
1	С	66	CRO	N2-CA2-CB2-CG2
1	D	66	CRO	N1-CA1-CB1-CG1
1	D	66	CRO	N1-CA1-CB1-OG1

There are no ring outliers.

2 monomers are involved in 6 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
1	В	66	CRO	4	0
1	D	66	CRO	2	0

5.5 Carbohydrates (i)

There are no oligosaccharides 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)

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^{th} 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 $>$	$\# \mathrm{RSRZ}{>}2$	$OWAB(Å^2)$	Q < 0.9
1	A	$224/236 \ (94\%)$	-0.36	2 (0%) 81 82	14, 22, 31, 56	0
1	В	$224/236 \ (94\%)$	-0.10	5 (2%) 62 64	16, 25, 42, 62	0
1	С	$224/236 \ (94\%)$	-0.14	15 (6%) 25 27	12, 22, 49, 65	0
1	D	$224/236 \ (94\%)$	0.03	7 (3%) 51 53	17, 28, 45, 65	0
All	All	896/944 (94%)	-0.14	29 (3%) 50 52	12, 24, 43, 65	0

The worst 5 of 29 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	D	229	ILE	7.6
1	С	174	GLY	6.1
1	В	229	ILE	4.8
1	В	228	GLY	4.5
1	С	190	ASP	4.1

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	$\mathbf{B} ext{-}\mathbf{factors}(\mathbf{\mathring{A}}^2)$	Q<0.9
1	CRO	В	66	22/23	0.82	0.15	17,22,24,25	0
1	CRO	A	66	22/23	0.89	0.11	17,22,24,25	0
1	CRO	D	66	22/23	0.90	0.09	17,22,24,25	0
1	CRO	С	66	22/23	0.93	0.08	17,22,24,25	0



6.3 Carbohydrates (i)

There are no monosaccharides in this entry.

6.4 Ligands (i)

There are no ligands in this entry.

6.5 Other polymers (i)

There are no such residues in this entry.

