

# Full wwPDB X-ray Structure Validation Report (i)

#### Apr 26, 2023 - 10:13 pm BST

PDB ID	:	8BRQ
Title	:	Crystal structure of a surface entropy reduction variant of penicillin G acylase
		from Bacillaceae i. s. sp. FJAT-27231
Authors	:	Wichmann, J.; Mayer, J.; Mattes, H.; Lukat, P.; Blankenfeldt, W.;
		Biedendieck, R.
Deposited on	:	2022-11-23
Resolution	:	1.63  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 (1)) were used in the production of this report:

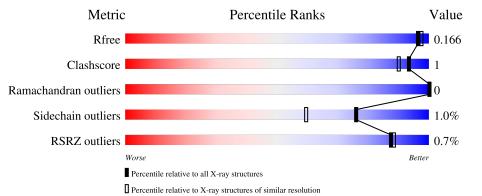
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as $541$ be (2020)
Xtriage (Phenix)	:	1.13
$\mathrm{EDS}$	:	2.32.2
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.32.2

## 1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:  $X\text{-}RAY \, DIFFRACTION$ 

The reported resolution of this entry is 1.63 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	$\begin{array}{c} \textbf{Whole archive} \\ (\#\textbf{Entries}) \end{array}$	${f Similar\ resolution}\ (\#{ m Entries,\ resolution\ range}({ m \AA}))$
$R_{free}$	130704	3122(1.66-1.62)
Clashscore	141614	3268(1.66-1.62)
Ramachandran outliers	138981	3215 (1.66-1.62)
Sidechain outliers	138945	3215 (1.66-1.62)
RSRZ outliers	127900	3079 (1.66-1.62)

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	А	212	<sup>2%</sup> 89%	•	8%
2	В	538	94%		• •



#### 8BRQ

## 2 Entry composition (i)

There are 6 unique types of molecules in this entry. The entry contains 12726 atoms, of which 5854 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 Penicillin G acylase.

Mol	Chain	Residues			Atoms	5			ZeroOcc	AltConf	Trace
1	А	196	Total 3282	C 1049	H 1619	N 281	O 326	${f S}{7}$	0	12	0

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
А	201	ALA	LYS	engineered mutation	UNP A0A0K9H482
А	202	ALA	LYS	engineered mutation	UNP A0A0K9H482
А	203	ALA	GLU	engineered mutation	UNP A0A0K9H482

• Molecule 2 is a protein called Penicillin G acylase.

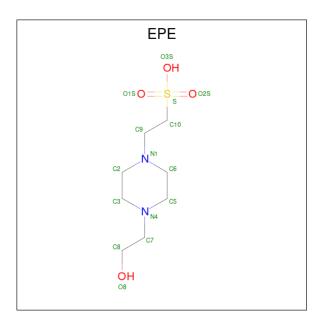
Mol	Chain	Residues			Atom	s			ZeroOcc	AltConf	Trace
2	В	525	Total 8502	C 2773	H 4188	N 733	O 797	S 11	0	22	0

There are 6 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
В	135	ALA	LYS	engineered mutation	UNP A0A0K9H482
В	136	ALA	GLU	engineered mutation	UNP A0A0K9H482
В	137	ALA	LYS	engineered mutation	UNP A0A0K9H482
В	403	ALA	LYS	engineered mutation	UNP A0A0K9H482
В	404	ALA	GLU	engineered mutation	UNP A0A0K9H482
В	405	ALA	GLU	engineered mutation	UNP A0A0K9H482

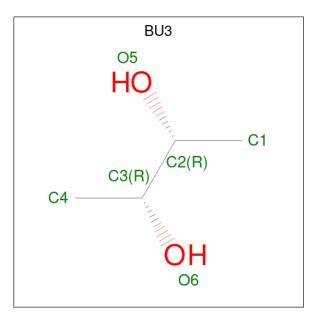
• Molecule 3 is 4-(2-HYDROXYETHYL)-1-PIPERAZINE ETHANESULFONIC ACID (three-letter code: EPE) (formula:  $C_8H_{18}N_2O_4S$ ).





Mol	Chain	Residues		A	ton	ıs			ZeroOcc	AltConf
3	В	1	Total 32	C 8	Н 17	N 2	0 4	S 1	0	0

• Molecule 4 is (R,R)-2,3-BUTANEDIOL (three-letter code: BU3) (formula:  $C_4H_{10}O_2$ ).



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	В	1	$\begin{array}{cccc} \text{Total}  \text{C}  \text{H}  \text{O} \\ 16  4  10  2 \end{array}$	0	0
4	В	1	Total         C         H         O           16         4         10         2	0	0
4	В	1	Total         C         H         O           16         4         10         2	0	0



• Molecule 5 is CALCIUM ION (three-letter code: CA) (formula: Ca).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	В	2	Total Ca 2 2	0	0

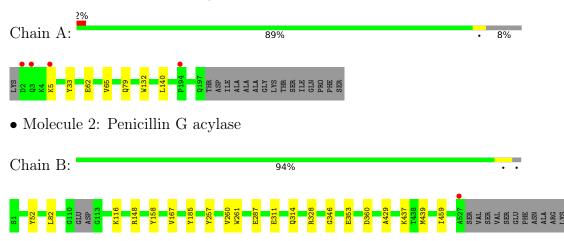
• Molecule 6 is water.

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
6	А	231	Total O 231 231	0	0
6	В	629	Total         O           629         629	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: Penicillin G acylase



## 4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants	61.25Å 102.11Å 140.08Å	Depositor
a, b, c, $\alpha$ , $\beta$ , $\gamma$	$90.00^{\circ}$ $90.00^{\circ}$ $90.00^{\circ}$	Depositor
Resolution (Å)	52.53 - 1.63	Depositor
Resolution (A)	82.51 - 1.63	EDS
% Data completeness	99.4 (52.53-1.63)	Depositor
(in resolution range)	99.4 (82.51-1.63)	EDS
R <sub>merge</sub>	0.09	Depositor
R <sub>sym</sub>	(Not available)	Depositor
$< I/\sigma(I) > 1$	$2.29 (at 1.63 \text{\AA})$	Xtriage
Refinement program	PHENIX 1.20-4459	Depositor
D D.	0.143 , $0.168$	Depositor
$R, R_{free}$	0.143 , $0.166$	DCC
$R_{free}$ test set	5451 reflections $(4.96\%)$	wwPDB-VP
Wilson B-factor $(Å^2)$	19.6	Xtriage
Anisotropy	0.379	Xtriage
Bulk solvent $k_{sol}(e/Å^3), B_{sol}(Å^2)$	0.35, 45.0	EDS
L-test for twinning <sup>2</sup>	$ < L >=0.48, < L^2>=0.31$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
$F_o, F_c$ correlation	0.97	EDS
Total number of atoms	12726	wwPDB-VP
Average B, all atoms $(Å^2)$	29.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 3.66% of the height of the origin peak. No significant pseudotranslation is detected.

<sup>&</sup>lt;sup>2</sup>Theoretical 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.



<sup>&</sup>lt;sup>1</sup>Intensities 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: BU3, CA, EPE

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		lengths	Bond angles		
	Unam	RMSZ	# Z  > 5	RMSZ	# Z  > 5	
1	А	0.48	0/1756	0.66	0/2361	
2	В	0.46	0/4523	0.63	0/6110	
All	All	0.46	0/6279	0.64	0/8471	

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	А	1663	1619	1557	3	0
2	В	4314	4188	4093	12	0
3	В	15	17	18	0	0
4	В	18	30	30	0	0
5	В	2	0	0	0	0
6	А	231	0	0	1	0
6	В	629	0	0	6	0
All	All	6872	5854	5698	16	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 1.



Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
2:B:287:GLU:OE1	6:B:701:HOH:O	2.15	0.65
2:B:311:GLU:OE2	2:B:328:ARG:NH2	2.34	0.59
2:B:167[B]:VAL:HG12	2:B:185:TYR:CE1	2.39	0.58
2:B:360:ASP:OD2	6:B:702:HOH:O	2.18	0.56
2:B:148:ARG:HD3	6:B:990:HOH:O	2.10	0.51
2:B:82:LEU:O	6:B:704:HOH:O	2.20	0.50
2:B:353:GLU:OE1	6:B:703:HOH:O	2.19	0.49
1:A:132:TRP:CZ3	1:A:140[A]:LEU:HD11	2.49	0.48
2:B:439[A]:MET:SD	2:B:459[A]:ILE:HG13	2.54	0.48
2:B:260:VAL:HG23	2:B:261:TRP:CD1	2.50	0.47
1:A:62[A]:GLU:HA	1:A:65:VAL:HG23	1.97	0.46
2:B:346:GLY:HA3	2:B:429:ALA:O	2.19	0.42
2:B:314:GLN:NE2	6:B:707:HOH:O	2.32	0.41
1:A:79:GLN:H	1:A:79:GLN:CD	2.25	0.41

All (16) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

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	ntiles
1	А	206/212~(97%)	202~(98%)	4(2%)	0	100	100
2	В	543/538~(101%)	528 (97%)	15 (3%)	0	100	100
All	All	749/750~(100%)	730~(98%)	19 (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 Outliers		Percentiles		
1	А	184/186~(99%)	182~(99%)	2(1%)	73 55		
2	В	454/451 (101%)	449 (99%)	5 (1%)	73 55		
All	All	638/637~(100%)	631 (99%)	7 (1%)	76 55		

All (7) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	А	5	LYS
1	А	33	TYR
2	В	116	LYS
2	В	158	TYR
2	В	257	TYR
2	В	437[A]	LYS
2	В	437[B]	LYS

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)

There are no non-standard protein/DNA/RNA residues in this entry.

### 5.5 Carbohydrates (i)

There are no monosaccharides in this entry.



### 5.6 Ligand geometry (i)

Of 6 ligands modelled in this entry, 2 are monoatomic - leaving 4 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	Res Link Bond lengths			Bond angles			
	туре	Unam	nes		Counts	RMSZ	# Z >2	Counts	RMSZ	# Z  > 2
4	BU3	В	602	-	$4,\!5,\!5$	0.31	0	$6,\!6,\!6$	0.62	0
3	EPE	В	601	-	$15,\!15,\!15$	0.68	1 (6%)	18,20,20	1.17	2 (11%)
4	BU3	В	604	-	4,5,5	0.22	0	6,6,6	0.50	0
4	BU3	В	603	-	4,5,5	0.27	0	6,6,6	0.50	0

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
4	BU3	В	602	-	-	0/4/4/4	-
3	EPE	В	601	-	-	2/9/19/19	0/1/1/1
4	BU3	В	604	-	-	0/4/4/4	-
4	BU3	В	603	-	-	0/4/4/4	-

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	$\operatorname{Ideal}(\operatorname{\AA})$
3	В	601	EPE	C10-S	2.23	1.80	1.77

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
3	В	601	EPE	C5-N4-C3	3.25	116.15	108.83
3	В	601	EPE	C9-N1-C2	-2.29	105.38	111.23

There are no chirality outliers.

All (2) torsion outliers are listed below:



Mol	Chain	Res	Type	Atoms
3	В	601	EPE	C8-C7-N4-C3
3	В	601	EPE	C8-C7-N4-C5

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,  $95^{th}$  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	$\langle RSRZ \rangle$	#RSRZ>2	$OWAB(Å^2)$	Q<0.9
1	А	196/212~(92%)	-0.55	4 (2%) 65 65	15, 22, 52, 84	0
2	В	525/538~(97%)	-0.62	1 (0%) 95 94	13, 22, 49, 77	0
All	All	721/750~(96%)	-0.60	5 (0%) 87 88	13, 22, 49, 84	0

All (5) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
2	В	527	ALA	3.5
1	А	3	GLN	3.4
1	А	5	LYS	3.2
1	А	194	PRO	2.2
1	А	2	ASP	2.0

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

There are no non-standard protein/DNA/RNA residues in this entry.

### 6.3 Carbohydrates (i)

There are no monosaccharides 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	RSR	$\mathbf{B} ext{-factors}(\mathrm{\AA}^2)$	Q < 0.9
4	BU3	В	604	6/6	0.86	0.19	38,50,61,71	0
4	BU3	В	603	6/6	0.90	0.11	30,43,55,61	0
4	BU3	В	602	6/6	0.90	0.17	28,37,53,53	0
3	EPE	В	601	15/15	0.97	0.08	19,27,59,62	32
5	CA	В	606	1/1	0.97	0.07	25,25,25,25	1
5	CA	В	605	1/1	1.00	0.07	$15,\!15,\!15,\!15$	0

## 6.5 Other polymers (i)

There are no such residues in this entry.

