

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

#### Aug 28, 2023 - 03:31 AM EDT

PDB ID	:	3K4K
Title	:	Pyranose 2-oxidase F454N mutant
Authors	:	Divne, C.; Tan, T.C.
Deposited on		
Resolution	:	1.60  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:

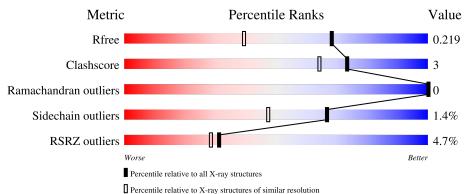
Xtriage (Phenix) EDS buster-report Percentile statistics Refmac CCP4 Ideal geometry (proteins) Ideal geometry (DNA, RNA)	::	20191225.v01 (using entries in the PDB archive December 25th 2019) 5.8.0158 7.0.044 (Gargrove) Engh & Huber (2001) Parkinson et al. (1996)
Ideal geometry (DNA, RNA) Validation Pipeline (wwPDB-VP)		Parkinson et al. (1996) 2.35

# 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 1.60 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	3398 (1.60-1.60)
Clashscore	141614	3665(1.60-1.60)
Ramachandran outliers	138981	3564 (1.60-1.60)
Sidechain outliers	138945	3563 (1.60-1.60)
RSRZ outliers	127900	3321 (1.60-1.60)

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							
			4%							
1	А	623	85%	7%	8%					



#### 3K4K

# 2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 5020 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.

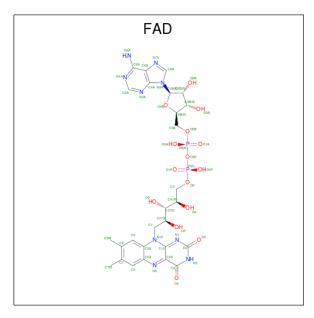
• Molecule 1 is a protein called Pyranose 2-oxidase.

Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	Trace	
1	А	575	Total 4531	C 2858	N 777	0 872	S 24	0	0	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
А	454	ASN	PHE	engineered mutation	UNP Q7ZA32

• Molecule 2 is FLAVIN-ADENINE DINUCLEOTIDE (three-letter code: FAD) (formula:  $\rm C_{27}H_{33}N_9O_{15}P_2).$ 



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	
2	А	1	Total 53	С 27			Р 2	0	0

• Molecule 3 is water.

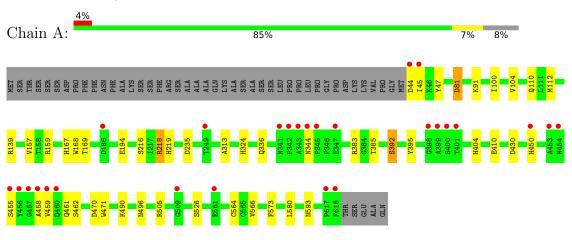


Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	А	436	Total O 436 436	0	0



# 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: Pyranose 2-oxidase



## 4 Data and refinement statistics (i)

Property	Value	Source	
Space group	P 42 21 2	Depositor	
Cell constants	102.06Å 102.06Å 119.69Å	Depositor	
a, b, c, $\alpha$ , $\beta$ , $\gamma$	$90.00^{\circ}$ $90.00^{\circ}$ $90.00^{\circ}$	Depositor	
Resolution (Å)	30.00 - 1.60	Depositor	
Resolution (A)	28.31 - 1.60	EDS	
% Data completeness	99.9 (30.00-1.60)	Depositor	
(in resolution range)	99.9 (28.31-1.60)	EDS	
R <sub>merge</sub>	(Not available)	Depositor	
R <sub>sym</sub>	0.10	Depositor	
$< I/\sigma(I) > 1$	$2.60 (at 1.60 \text{\AA})$	Xtriage	
Refinement program	REFMAC 5.2.0019	Depositor	
B B.	0.185 , $0.213$	Depositor	
$R, R_{free}$	0.193 , $0.219$	DCC	
$R_{free}$ test set	1961 reflections $(2.35\%)$	wwPDB-VP	
Wilson B-factor $(Å^2)$	14.9	Xtriage	
Anisotropy	0.072	Xtriage	
Bulk solvent $k_{sol}(e/Å^3), B_{sol}(Å^2)$	0.38, 46.3	EDS	
L-test for twinning <sup>2</sup>	$ < L >=0.42, < L^2>=0.25$	Xtriage	
Estimated twinning fraction	No twinning to report.	Xtriage	
$F_o, F_c$ correlation	0.95	EDS	
Total number of atoms	5020	wwPDB-VP	
Average B, all atoms $(Å^2)$	16.0	wwPDB-VP	

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 4.13% 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: FAD

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		
	Mol Chain		# Z  > 5	RMSZ	# Z  > 5	
1	А	0.91	0/4646	0.93	11/6318~(0.2%)	

There are no bond length outliers.

All (11) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
1	А	139	ARG	NE-CZ-NH2	-9.57	115.51	120.30
1	А	139	ARG	NE-CZ-NH1	8.47	124.54	120.30
1	А	505	ARG	NE-CZ-NH1	8.32	124.46	120.30
1	А	81	ASP	CB-CG-OD1	-7.94	111.16	118.30
1	А	81	ASP	CB-CG-OD2	7.32	124.89	118.30
1	А	470	ASP	CB-CG-OD2	-6.84	112.15	118.30
1	А	235	ASP	CB-CG-OD2	-6.28	112.65	118.30
1	А	470	ASP	CB-CG-OD1	5.98	123.68	118.30
1	А	505	ARG	NE-CZ-NH2	-5.30	117.65	120.30
1	А	218	ARG	NE-CZ-NH1	5.10	122.85	120.30
1	А	159	ARG	NE-CZ-NH1	5.02	122.81	120.30

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	А	4531	0	4376	30	0
2	А	53	0	29	0	0
3	А	436	0	0	10	0
All	All	5020	0	4405	30	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 3.

All (30) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic	Clash
	At0111-2	distance (Å)	overlap (Å)
1:A:110:GLN:HE21	1:A:167:HIS:HD1	1.23	0.82
1:A:459:VAL:O	1:A:462:SER:HB3	1.81	0.81
1:A:395:TYR:OH	1:A:410:GLU:HG2	1.93	0.68
1:A:404:HIS:HE1	3:A:1136:HOH:O	1.74	0.68
1:A:459:VAL:O	1:A:462:SER:CB	2.47	0.62
1:A:91:LYS:HD2	1:A:100:ILE:HD11	1.83	0.60
1:A:194:GLU:CG	3:A:1395:HOH:O	2.49	0.60
1:A:44:ASP:N	1:A:47:TYR:HH	1.98	0.60
1:A:81:ASP:HB2	3:A:1341:HOH:O	2.02	0.59
1:A:81:ASP:OD1	1:A:81:ASP:C	2.41	0.59
1:A:392:SER:HB3	3:A:1229:HOH:O	2.04	0.58
1:A:81:ASP:CA	3:A:1341:HOH:O	2.52	0.57
1:A:564:CYS:HG	1:A:573:PHE:HE2	1.55	0.55
1:A:218:ARG:HG3	1:A:430:ASP:OD2	2.14	0.47
1:A:383:ARG:HD2	3:A:1348:HOH:O	2.15	0.46
1:A:216:SER:HB3	1:A:219:HIS:HB3	1.98	0.45
1:A:194:GLU:CD	3:A:1395:HOH:O	2.55	0.45
1:A:496:ASN:O	1:A:496:ASN:CG	2.55	0.45
1:A:47:TYR:O	1:A:313:ALA:HA	2.17	0.44
1:A:458:ALA:HA	1:A:461:GLN:OE1	2.18	0.44
1:A:169:THR:HG22	1:A:169:THR:O	2.18	0.43
1:A:218:ARG:HD2	3:A:1145:HOH:O	2.18	0.43
1:A:194:GLU:HG2	3:A:1395:HOH:O	2.14	0.43
1:A:471:TRP:CH2	1:A:526:SER:HA	2.54	0.43
1:A:81:ASP:HA	3:A:1341:HOH:O	2.17	0.42
1:A:336:GLN:NE2	1:A:344:ASN:O	2.52	0.42
1:A:45:ILE:C	1:A:45:ILE:HD12	2.40	0.42
1:A:157:VAL:HG21	1:A:324:HIS:HE1	1.85	0.41
1:A:566:VAL:CG1	1:A:580:LEU:HD12	2.51	0.41
1:A:104:VAL:HG11	1:A:455:SER:HB3	2.03	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	lysed Favoured Allo		Outliers		
1	А	573/623~(92%)	556~(97%)	17 (3%)	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	Analysed Rotameric		Percentiles	
1	А	503/542~(93%)	496 (99%)	7~(1%)	67 47	

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

Mol	Chain	Res	Type
1	А	112	MET
1	А	168	TRP
1	А	385	THR
1	А	392	SER
1	А	450	HIS
1	А	490	LYS
1	А	593	ASN

Sometimes side chains can be flipped to improve hydrogen bonding and reduce clashes. All (4) such side chains are listed below:



Mol	Chain	Res	Type
1	А	263	GLN
1	А	404	HIS
1	А	443	HIS
1	А	460	GLN

#### 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)

1 ligand 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 Lin	Dec	Tiple	B	ond leng	gths	B	ond ang	gles
IVIOI	туре		LIIIK	Counts	RMSZ	# Z  > 2	Counts	RMSZ	# Z >2	
2	FAD	А	801	1	53,58,58	1.68	10 (18%)	68,89,89	1.86	18 (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
2	FAD	А	801	1	-	3/30/50/50	0/6/6/6



Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	А	801	FAD	C2A-N3A	4.69	1.39	1.32
2	А	801	FAD	C5'-C4'	3.82	1.57	1.51
2	А	801	FAD	C4X-N5	3.65	1.37	1.30
2	А	801	FAD	O4B-C4B	-3.32	1.37	1.45
2	А	801	FAD	C9-C8	-3.26	1.34	1.39
2	А	801	FAD	C2B-C1B	-3.15	1.49	1.53
2	А	801	FAD	C5X-N5	-3.11	1.33	1.39
2	А	801	FAD	O3B-C3B	-2.67	1.36	1.43
2	А	801	FAD	C6-C5X	2.59	1.44	1.40
2	А	801	FAD	C10-N1	2.58	1.38	1.33

All (10) bond length outliers are listed below:

All (18) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
2	А	801	FAD	C4-C4X-N5	5.47	126.01	118.23
2	А	801	FAD	O3B-C3B-C4B	4.51	124.08	111.05
2	А	801	FAD	C5A-C6A-N6A	4.17	126.69	120.35
2	А	801	FAD	C4X-C4-N3	3.50	122.09	113.19
2	А	801	FAD	C4-N3-C2	-3.35	119.45	125.64
2	А	801	FAD	O2B-C2B-C1B	3.10	122.29	110.85
2	А	801	FAD	O4-C4-C4X	-2.98	118.68	126.60
2	А	801	FAD	C9-C8-C7	2.88	123.80	119.67
2	А	801	FAD	C6-C5X-C9A	2.77	122.86	118.94
2	А	801	FAD	O2'-C2'-C1'	-2.76	103.12	109.80
2	А	801	FAD	C10-C4X-N5	-2.48	119.58	124.86
2	А	801	FAD	C8M-C8-C9	-2.42	115.01	119.49
2	А	801	FAD	C4A-C5A-N7A	-2.40	106.90	109.40
2	А	801	FAD	C5X-C6-C7	-2.22	116.63	120.71
2	А	801	FAD	N3A-C2A-N1A	-2.22	125.22	128.68
2	А	801	FAD	C5'-C4'-C3'	-2.15	108.05	112.20
2	А	801	FAD	C10-N1-C2	2.03	120.97	116.90
2	А	801	FAD	O2B-C2B-C3B	2.02	118.35	111.82

There are no chirality outliers.

All (3) torsion outliers are listed below:

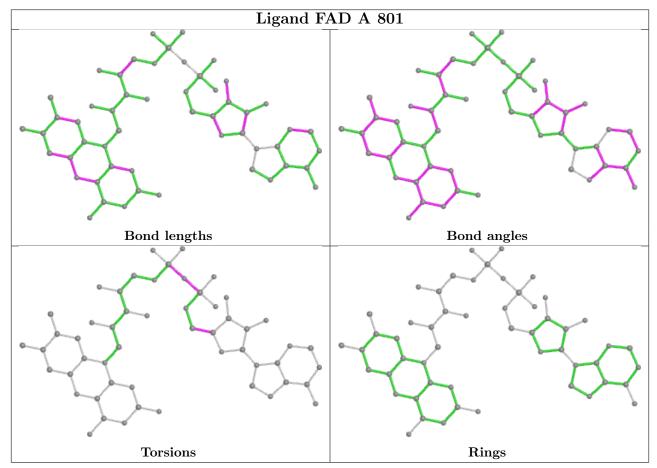
Mol	Chain	Res	Type	Atoms
2	А	801	FAD	PA-O3P-P-O5'
2	А	801	FAD	O4B-C4B-C5B-O5B
2	А	801	FAD	P-O3P-PA-O2A



There are no ring outliers.

No monomer is involved in short contacts.

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less then 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.



### 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 $>$	#RSRZ>2		$\mathbf{OWAB}(\mathbf{A}^2)$	Q<0.9
1	А	575/623~(92%)	0.15	27 (4%) 3	1 28	8, 13, 27, 43	0

All (27) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ	
1	А	459	VAL	12.7	
1	А	458 ALA		12.0	
1	А	343 ALA		6.7	
1	А	456	TYR	6.1	
1	А	341	ASN	5.5	
1	А	457	GLY	5.0	
1	А	344	ASN	4.9	
1	А	618	PHE	4.9	
1	А	400	SER	4.4	
1	А	453	ALA	4.4	
1	А	342	PRO	4.2	
1	А	401	THR	4.1	
1	А	455	SER	4.0	
1	А	345	PRO	3.6	
1	А	460	GLN	3.4	
1	А	44	ASP	3.2	
1	А	45	ILE	3.1	
1	А	249	THR	2.7	
1	А	617	PRO	2.6	
1	А	454	ASN	2.4	
1	А	347	GLU	2.3	
1	А	399	ALA	2.3	
1	А	398	GLY	2.3	
1	А	509	GLY	2.2	
1	А	561	GLU	2.1	
1	А	186	ASP	2.1	
1	А	450	HIS	2.1	



## 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)

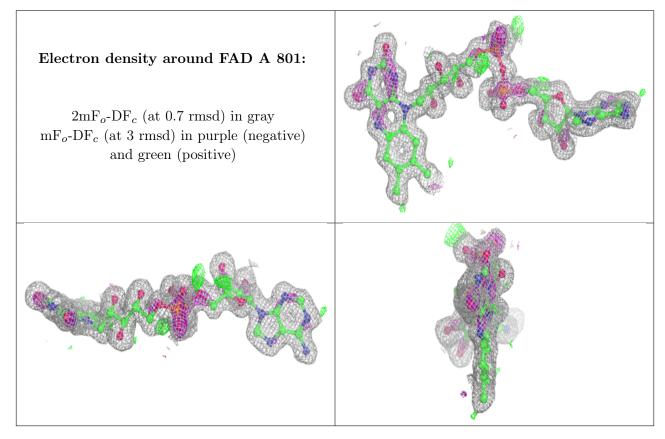
There are no monosaccharides in this entry.

## 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	${f B} ext{-factors}({ m \AA}^2)$	Q < 0.9
2	FAD	А	801	53/53	0.97	0.07	$9,\!11,\!14,\!16$	0

The following is a graphical depiction of the model fit to experimental electron density of all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the geometry validation Tables will also be included. Each fit is shown from different orientation to approximate a three-dimensional view.





## 6.5 Other polymers (i)

There are no such residues in this entry.

