

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

Jan 5, 2024 – 12:43 am GMT

PDB ID	:	4UNE
Title	:	Human insulin B26Phe mutant crystal structure
Authors	:	Zakova, L.; Klevtikova, E.; Lepsik, M.; Collinsova, M.; Watson, C.J.; Turken-
		burg, J.P.; Jiracek, J.; Brzozowski, A.M.
Deposited on	:	2014-05-28
Resolution	:	1.59  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:

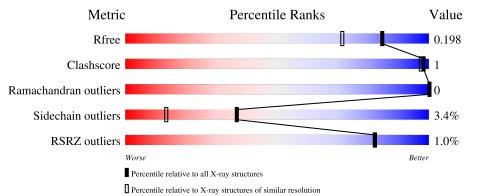
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as $541$ be (2020)
Xtriage (Phenix)	:	1.13
$\mathrm{EDS}$	:	2.36
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36

## 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 1.59 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	
1	А	21	95%	5%
1	С	21	95%	5%
2	В	30	3%	
2	D	30	87%	10% •

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
3	SO4	D	1030	-	Х	-	-



#### 4UNE

## 2 Entry composition (i)

There are 4 unique types of molecules in this entry. The entry contains 963 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 INSULIN A CHAIN.

Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace		
1	Δ	91	Total	С	Ν	Ο	S	0	1	0
1		21	166	101	25	36	4	0	T	0
1	С	C 21	Total	С	Ν	Ο	$\mathbf{S}$	0	0	0
1			163	99	25	35	4			0

• Molecule 2 is a protein called INSULIN B CHAIN.

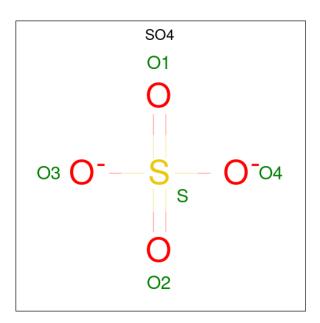
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace		
9	В	30	Total	С	Ν	Ο	S	0	0	0
		- 50	234	156	40	36	2	0		
0	Л	29	Total	С	Ν	Ο	S	0	0	0
		29	229	152	39	36	2			U

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
В	26	PHE	TYR	engineered mutation	UNP P01308
D	26	PHE	TYR	engineered mutation	UNP P01308

• Molecule 3 is SULFATE ION (three-letter code: SO4) (formula: O<sub>4</sub>S).





Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	В	1	$\begin{array}{ccc} \text{Total} & \text{O} & \text{S} \\ 5 & 4 & 1 \end{array}$	0	0
3	D	1	$\begin{array}{ccc} \text{Total} & \text{O} & \text{S} \\ 5 & 4 & 1 \end{array}$	0	0

• Molecule 4 is water.

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	А	40	Total O 40 40	0	0
4	В	46	Total         O           46         46	0	0
4	С	38	Total         O           38         38	0	0
4	D	37	$\begin{array}{cc} \text{Total} & \text{O} \\ 37 & 37 \end{array}$	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.

Chain A:	95%	5%
N2 1		
• Molecule 1: INSULIN A CHAIN		
Chain C:	95%	5%
N21 N21		
• Molecule 2: INSULIN B CHAIN		
Chain B:	100%	
• Molecule 2: INSULIN B CHAIN		
Chain D:	87%	10% •
F1 K29 THR		

• Molecule 1: INSULIN A CHAIN



## 4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants	43.89Å 45.76Å 51.31Å	Denesiten
a, b, c, $\alpha$ , $\beta$ , $\gamma$	$90.00^{\circ}$ $90.00^{\circ}$ $90.00^{\circ}$	Depositor
Resolution (Å)	34.15 - 1.59	Depositor
Resolution (A)	34.15 - 1.59	EDS
% Data completeness	98.4(34.15-1.59)	Depositor
(in resolution range)	98.4(34.15-1.59)	EDS
R <sub>merge</sub>	0.08	Depositor
R <sub>sym</sub>	(Not available)	Depositor
$< I/\sigma(I) > 1$	$2.57 (at 1.58 \text{\AA})$	Xtriage
Refinement program	REFMAC 5.8.0033	Depositor
B B.	0.151 , $0.180$	Depositor
$R, R_{free}$	0.165 , $0.198$	DCC
$R_{free}$ test set	722 reflections $(5.02\%)$	wwPDB-VP
Wilson B-factor $(Å^2)$	10.8	Xtriage
Anisotropy	0.403	Xtriage
Bulk solvent $k_{sol}(e/Å^3)$ , $B_{sol}(Å^2)$	0.34 , $51.1$	EDS
L-test for twinning <sup>2</sup>	$< L >=0.48, < L^2>=0.31$	Xtriage
Estimated twinning fraction	0.028 for k,h,-l	Xtriage
$F_o, F_c$ correlation	0.96	EDS
Total number of atoms	963	wwPDB-VP
Average B, all atoms $(Å^2)$	13.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The analyses of the Patterson function reveals a significant off-origin peak that is 61.16 % of the origin peak, indicating pseudo-translational symmetry. The chance of finding a peak of this or larger height randomly in a structure without pseudo-translational symmetry is equal to 1.3651e-05. The detected translational NCS is most likely also responsible for the elevated intensity ratio.

<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: SO4

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		
	Chain	RMSZ	# Z  > 5	RMSZ	# Z  > 5	
1	А	1.26	0/170	0.92	0/228	
1	С	1.23	0/164	0.91	0/220	
2	В	1.25	0/241	0.91	0/325	
2	D	1.16	0/236	1.01	0/317	
All	All	1.22	0/811	0.94	0/1090	

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
2	D	0	1

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
2	D	28	PRO	Peptide

### 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	166	0	154	0	0
1	С	163	0	149	0	0
2	В	234	0	228	0	0
2	D	229	0	219	1	0
3	В	5	0	0	0	0
3	D	5	0	0	0	0
4	А	40	0	0	0	0
4	В	46	0	0	0	1
4	С	38	0	0	0	1
4	D	37	0	0	1	0
All	All	963	0	750	1	1

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.

All (1) 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)	
2:D:1:PHE:N	4:D:2001:HOH:O	2.29	0.43	

All (1) symmetry-related close contacts are listed below. The label for Atom-2 includes the symmetry operator and encoded unit-cell translations to be applied.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
4:B:2039:HOH:O	4:C:2001:HOH:O[3_554]	1.95	0.25

### 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	20/21~(95%)	18 (90%)	2(10%)	0	100	100
1	С	19/21~(90%)	18 (95%)	1 (5%)	0	100	100

Continued on next page...



Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles				
2	В	28/30~(93%)	28 (100%)	0	0	100	100			
2	D	27/30~(90%)	27 (100%)	0	0	100	100			
All	All	94/102~(92%)	91 (97%)	3 (3%)	0	100	100			

Continued from previous page...

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	21/20~(105%)	20~(95%)	1 (5%)	25 7	
1	С	20/20~(100%)	19~(95%)	1 (5%)	24 6	
2	В	24/26~(92%)	24 (100%)	0	100 100	
2	D	24/26~(92%)	23~(96%)	1 (4%)	30 9	
All	All	89/92~(97%)	86~(97%)	3~(3%)	37 13	

All (3) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	А	21	ASN
1	С	21	ASN
2	D	29	LYS

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

Mol	Chain	Res	Type
1	А	5	GLN
2	В	5	HIS
2	В	10	HIS



#### 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)

2 ligands 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).

Mol	Type	Chain	Res Link		B	ond leng	$\operatorname{gths}$	B	Sond ang	gles	
MIOI	туре	Chain	nes	nes	nes Link	Counts	RMSZ	# Z  > 2	Counts	RMSZ	# Z  > 2
3	SO4	D	1030	-	4,4,4	1.36	1 (25%)	$6,\!6,\!6$	2.14	4 (66%)	
3	SO4	В	1031	-	4,4,4	0.94	0	$6,\!6,\!6$	0.73	0	

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	D	1030	SO4	O2-S	2.48	1.59	1.46

All (4) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Ζ	$Observed(^{o})$	$Ideal(^{o})$
3	D	1030	SO4	O4-S-O2	3.03	125.11	109.31
3	D	1030	SO4	O3-S-O2	-2.86	94.39	109.31
3	D	1030	SO4	03-S-01	2.14	120.48	109.31
3	D	1030	SO4	02-S-01	-2.08	94.04	109.43



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,  $95^{th}$  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	$OWAB(Å^2)$	Q < 0.9
1	А	21/21~(100%)	-0.48	0 100 100	7, 10, 11, 21	0
1	С	21/21~(100%)	-0.56	0 100 100	8, 9, 12, 19	0
2	В	30/30~(100%)	-0.28	1 (3%) 46 43	8, 11, 20, 34	0
2	D	29/30~(96%)	-0.21	0 100 100	8, 12, 22, 23	0
All	All	101/102~(99%)	-0.36	1 (0%) 82 82	7, 10, 21, 34	0

All (1) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
2	В	30	THR	2.4

### 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	B-factors(Å <sup>2</sup> )	Q<0.9
3	SO4	В	1031	5/5	0.80	0.34	31,34,41,44	0

Continued on next page...



Continued from previous page...

Mol	Type	Chain	Res	Atoms	RSCC	RSR	$\mathbf{B} ext{-factors}(\mathrm{\AA}^2)$	Q<0.9
3	SO4	D	1030	5/5	0.96	0.10	16,20,25,29	0

## 6.5 Other polymers (i)

There are no such residues in this entry.

