W O R L D W I D E
wwPDB
P R O T E I N   D A T A   B A N K

Full wwPDB X-ray Structure Validation Report ⓘ

Feb 13, 2017 – 03:18 am GMT

PDB ID : 4UG8
Title : Structure of Bacillus subtilis Nitric Oxide Synthase in complex with 6-(5-((3
R,4R)-4-((6-azanyl-4-methyl-pyridin-2-yl)methyl)pyrrolidin-3- yl)oxypentyl)-
4-methyl-pyridin-2-amine
Authors : Holden, J.K.; Poulos, T.L.
Deposited on : 2015-03-22
Resolution : 1.89 Å(reported)

1 Overall quality at a glance ⓘ

The following experimental techniques were used to determine the structure:
X-RAY DIFFRACTION

The reported resolution of this entry is 1.89 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	Percentile Ranks	Value
Rfree		0.228
Clashscore		3
Ramachandran outliers		0
Sidechain outliers		2.3%
RSRZ outliers		45.3%

Worse   *Better*

▮ Percentile relative to all X-ray structures

▯ Percentile relative to X-ray structures of similar resolution
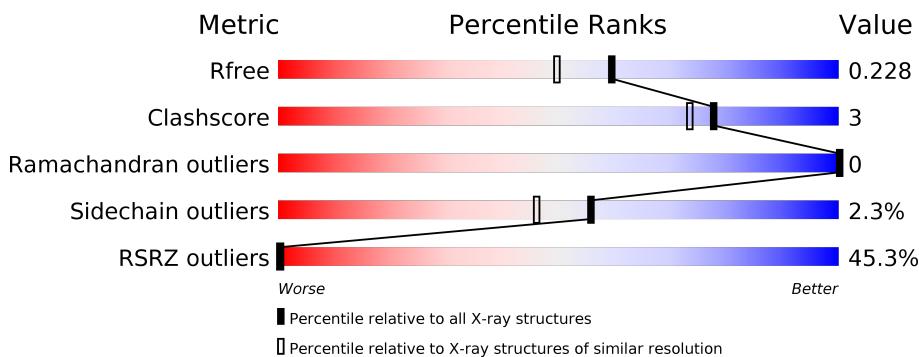
Metric	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
R_{free}	100719	7505 (1.90-1.86)
Clashscore	112137	8369 (1.90-1.86)
Ramachandran outliers	110173	8279 (1.90-1.86)
Sidechain outliers	110143	8280 (1.90-1.86)
RSRZ outliers	101464	7571 (1.90-1.86)

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 on the lower bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria. 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	363	45% — 90% / 10%
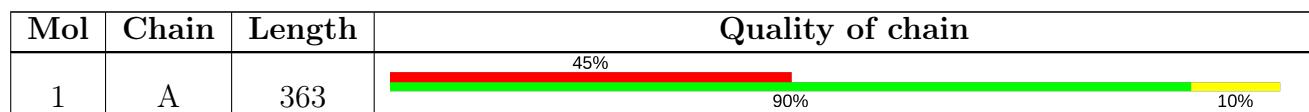
The following table lists non-polymeric compounds, carbohydrate monomers and non-standard residues in protein, DNA, RNA chains that are outliers for geometric or electron-density-fit criteria:

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
5	HW1	A	904	-	-	-	X

2 Entry composition ⓘ

There are 7 unique types of molecules in this entry. The entry contains 3279 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 NITRIC OXIDE SYNTHASE OXYGENASE.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	362	Total 2941	C 1873	N 507	O 553	S 8	0	0	0

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	25	ALA	GLU	ENGINEERED MUTATION	UNP O34453
A	26	ALA	GLU	ENGINEERED MUTATION	UNP O34453
A	316	ALA	GLU	ENGINEERED MUTATION	UNP O34453

- Molecule 2 is PROTOPORPHYRIN IX CONTAINING FE (three-letter code: HEM) (formula: $C_{34}H_{32}FeN_4O_4$).
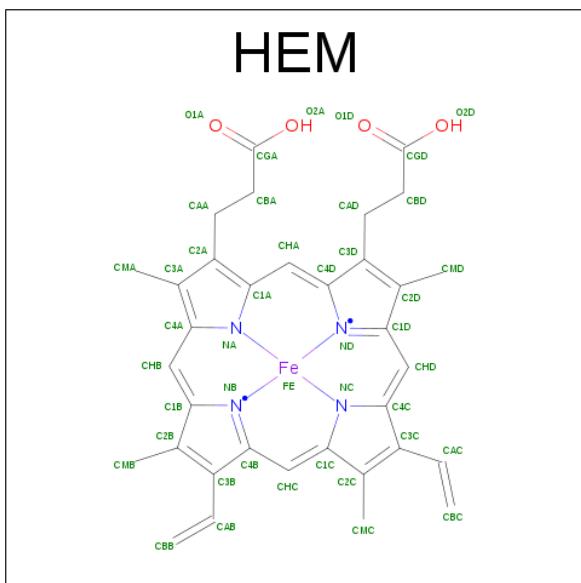
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
2	A	1	Total 43	C 34	Fe 1	N 4	O 4	0	0

- Molecule 3 is 5,6,7,8-TETRAHYDROBIOPTERIN (three-letter code: H4B) (formula: $C_9H_{15}N_5O_3$).
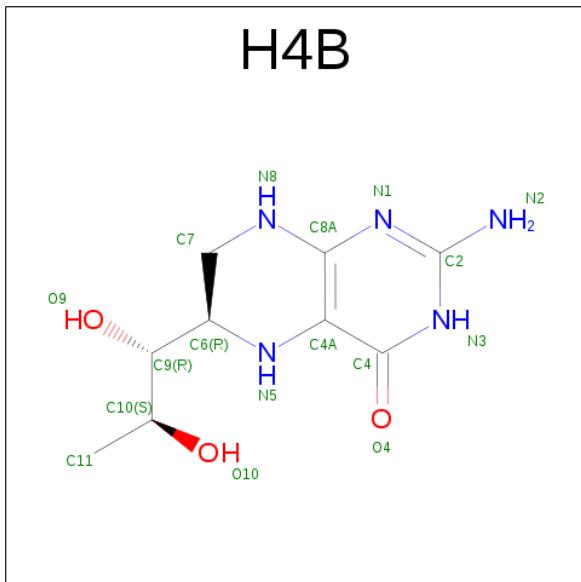
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
			Total	C	N	O		
3	A	1	17	9	5	3	0	0

- Molecule 4 is CHLORIDE ION (three-letter code: CL) (formula: Cl).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	Cl		
4	A	1	1	1	0	0

- Molecule 5 is 6-[5-({(3R,4R)-4-[(6-AMINO-4-METHYLPYRIDIN-2-YL)METHYL]PYRR OLIDIN-3-YL}OXY)PENTYL]-4-METHYLPYRIDIN-2-AMINE (three-letter code: HW1) (formula: $C_{22}H_{33}N_5O$).
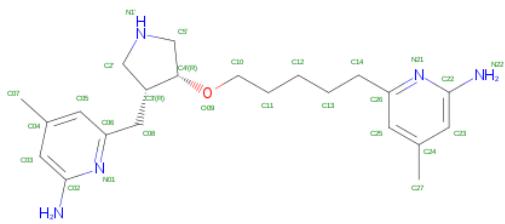
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
			Total	C	N	O		
5	A	1	28	22	5	1	0	0

- Molecule 6 is GLYCEROL (three-letter code: GOL) (formula: $C_3H_8O_3$).
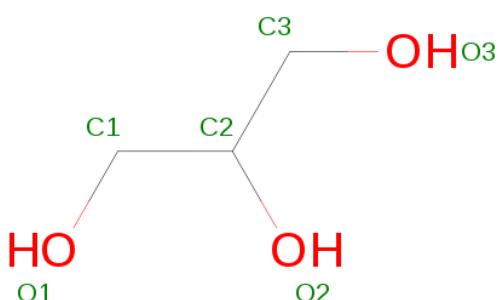
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
			Total	C	O		
6	A	1	6	3	3	0	0

- Molecule 7 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
7	A	243	Total 243	O 243	0	0

3 Residue-property plots ⓘ

These plots are drawn for all protein, RNA and DNA 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: NITRIC OXIDE SYNTHASE OXYGENASE
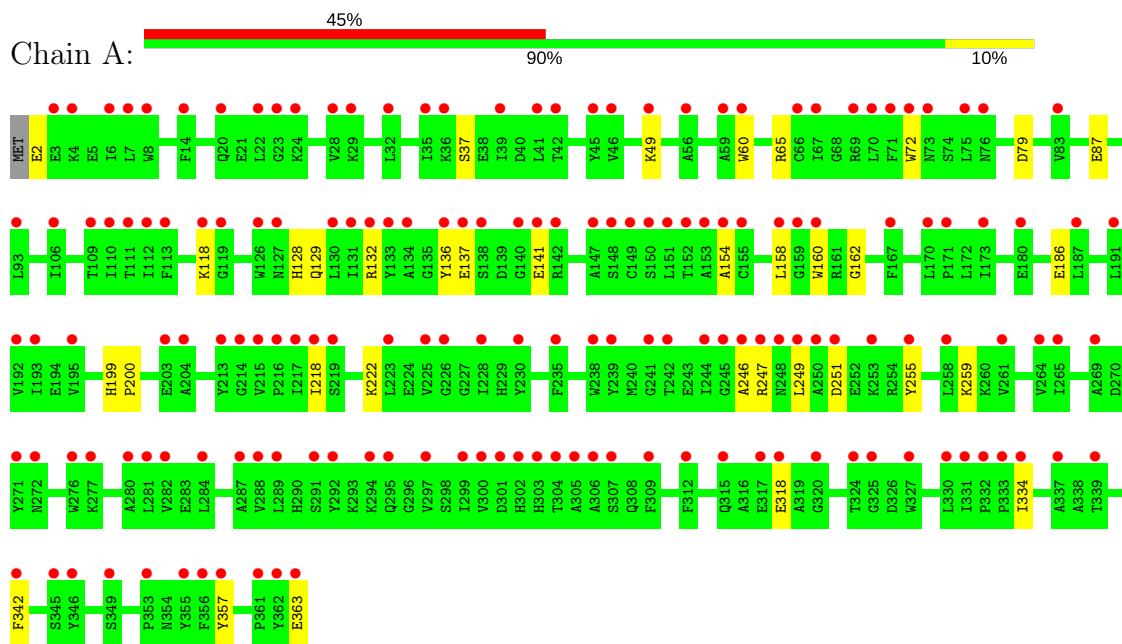
4   Data and refinement statistics ⓘ

Property	Value	Source
Space group	P 21 21 2	Depositor
Cell constants a, b, c, α, β, γ	80.55Å 95.19Å 62.77Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	37.09 – 1.89 37.09 – 1.89	Depositor EDS
% Data completeness (in resolution range)	80.8 (37.09-1.89) 80.8 (37.09-1.89)	Depositor EDS
R_{merge}	0.07	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	3.49 (at 1.89Å)	Xtriage
Refinement program	PHENIX (PHENIX.REFINE)	Depositor
R, R_{free}	0.187 , 0.228 0.186 , 0.228	Depositor DCC
R_{free} test set	1613 reflections (5.06%)	DCC
Wilson B-factor (Å^2)	25.8	Xtriage
Anisotropy	0.019	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.34 , 41.9	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	0.92	EDS
Total number of atoms	3279	wwPDB-VP
Average B, all atoms (Å^2)	38.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.43% 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 ⓘ

5.1 Standard geometry ⓘ

Bond lengths and bond angles in the following residue types are not validated in this section: GOL, HW1, H4B, HEM, CL

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	0.34	0/3017	0.49	0/4088

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 ⓘ

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	2941	0	2856	16	2
2	A	43	0	30	4	0
3	A	17	0	15	2	1
4	A	1	0	0	0	0
5	A	28	0	33	3	0
6	A	6	0	8	0	0
7	A	243	0	0	1	0
All	All	3279	0	2942	20	2

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 (20) 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:218:ILE:HD11	5:A:904:HW1:H25	1.76	0.66
1:A:246:ALA:HB2	1:A:334:ILE:HD12	1.83	0.59
1:A:60:TRP:CE3	1:A:72:TRP:HA	2.40	0.56
1:A:247:ARG:NH1	1:A:251:ASP:OD2	2.43	0.52
2:A:901:HEM:O1A	3:A:902:H4B:N3	2.43	0.51
1:A:222:LYS:NZ	7:A:2145:HOH:O	2.43	0.50
1:A:49:LYS:NZ	1:A:79:ASP:OD2	2.45	0.49
1:A:160:TRP:NE1	1:A:186:GLU:OE2	2.44	0.49
2:A:901:HEM:HBA1	5:A:904:HW1:H27	1.93	0.49
1:A:128:HIS:CE1	5:A:904:HW1:H18	2.49	0.48
1:A:154:ALA:O	1:A:158:LEU:HD12	2.15	0.47
1:A:65:ARG:NH1	1:A:357:TYR:OH	2.48	0.46
2:A:901:HEM:O1A	3:A:902:H4B:N2	2.45	0.46
1:A:136:TYR:OH	1:A:162:GLY:HA3	2.17	0.45
1:A:249:LEU:HA	1:A:255:TYR:HB2	1.98	0.45
2:A:901:HEM:HBB2	2:A:901:HEM:HHC	1.99	0.44
1:A:129:GLN:HG2	1:A:218:ILE:HD13	1.99	0.43
1:A:222:LYS:HE3	1:A:222:LYS:HB2	1.81	0.42
1:A:118:LYS:HE3	1:A:118:LYS:HB2	1.81	0.42
1:A:199:HIS:CG	1:A:200:PRO:HD2	2.56	0.41

All (2) 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)
1:A:342:PHE:O	3:A:902:H4B:O9[2_555]	2.00	0.20
1:A:87:GLU:OE2	1:A:259:LYS:NZ[3_556]	2.07	0.13

5.3 Torsion angles ⓘ

5.3.1 Protein backbone ⓘ

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	360/363 (99%)	350 (97%)	10 (3%)	0	100	100

There are no Ramachandran outliers to report.

5.3.2 Protein sidechains ⓘ

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	309/310 (100%)	302 (98%)	7 (2%)	56	46

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

Mol	Chain	Res	Type
1	A	2	GLU
1	A	37	SER
1	A	132	ARG
1	A	137	GLU
1	A	141	GLU
1	A	318	GLU
1	A	363	GLU

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains ⓘ

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

5.5 Carbohydrates ⓘ

There are no carbohydrates in this entry.

5.6 Ligand geometry ⓘ

Of 5 ligands modelled in this entry, 1 is 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	Link	Bond lengths			Bond angles		
					Counts	RMSZ	#$|Z| > 2$	Counts	RMSZ	#$|Z| > 2$
2	HEM	A	901	1	28,50,50	0.93	1 (3%)	17,82,82	1.28	2 (11%)
3	H4B	A	902	-	14,18,18	2.01	2 (14%)	12,26,26	2.02	4 (33%)
5	HW1	A	904	-	27,30,30	0.44	0	34,40,40	1.67	4 (11%)
6	GOL	A	905	-	5,5,5	0.20	0	5,5,5	0.40	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
2	HEM	A	901	1	-	0/6/54/54	0/0/8/8
3	H4B	A	902	-	-	0/8/17/17	0/2/2/2
5	HW1	A	904	-	-	0/13/23/23	0/3/3/3
6	GOL	A	905	-	-	0/4/4/4	0/0/0/0

All (3) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	901	HEM	C3B-C2B	-3.09	1.36	1.40
3	A	902	H4B	C4A-C8A	3.60	1.48	1.41
3	A	902	H4B	C4-C4A	6.15	1.48	1.41

All (10) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
5	A	904	HW1	C05-C06-N01	-3.35	119.28	122.91
5	A	904	HW1	C24-C25-C26	-3.19	118.26	120.26
2	A	901	HEM	CBD-CAD-C3D	-2.97	106.80	112.47
2	A	901	HEM	CBA-CAA-C2A	-2.57	107.56	112.48

Continued on next page...

Continued from previous page...

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	A	902	H4B	N2-C2-N1	2.01	120.45	117.24
3	A	902	H4B	C4-C4A-C8A	2.53	116.85	114.56
3	A	902	H4B	C2-N1-C8A	3.07	121.42	114.51
5	A	904	HW1	C22-N21-C26	4.28	121.20	118.17
3	A	902	H4B	C4-N3-C2	4.39	122.37	116.06
5	A	904	HW1	C02-N01-C06	5.53	122.08	118.17

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

3 monomers are involved in 7 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	901	HEM	4	0
3	A	902	H4B	2	1
5	A	904	HW1	3	0

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 ⓘ

6.1 Protein, DNA and RNA chains ⓘ

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, 95th 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	A	362/363 (99%)	1.98	164 (45%)	0	0	21, 33, 67, 92	0

All (164) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	118	LYS	5.0
1	A	356	PHE	4.7
1	A	288	VAL	4.5
1	A	35	ILE	4.4
1	A	334	ILE	4.3
1	A	213	TYR	4.3
1	A	137	GLU	4.3
1	A	119	GLY	4.2
1	A	155	CYS	4.0
1	A	141	GLU	3.9
1	A	41	LEU	3.8
1	A	173	ILE	3.7
1	A	333	PRO	3.7
1	A	59	ALA	3.7
1	A	355	TYR	3.7
1	A	66	CYS	3.7
1	A	217	ILE	3.7
1	A	60	TRP	3.6
1	A	29	LYS	3.6
1	A	363	GLU	3.6
1	A	239	TYR	3.6
1	A	160	TRP	3.5
1	A	39	ILE	3.5
1	A	72	TRP	3.5
1	A	158	LEU	3.5
1	A	289	LEU	3.5
1	A	312	PHE	3.4

Continued on next page...

Continued from previous page...

Mol	Chain	Res	Type	RSRZ
1	A	140	GLY	3.4
1	A	24	LYS	3.4
1	A	238	TRP	3.4
1	A	337	ALA	3.4
1	A	284	LEU	3.3
1	A	247	ARG	3.3
1	A	67	ILE	3.3
1	A	276	TRP	3.3
1	A	306	ALA	3.2
1	A	250	ALA	3.2
1	A	203	GLU	3.2
1	A	362	TYR	3.2
1	A	214	GLY	3.1
1	A	109	THR	3.1
1	A	23	GLY	3.1
1	A	151	LEU	3.1
1	A	215	VAL	3.1
1	A	242	THR	3.1
1	A	133	TYR	3.1
1	A	357	TYR	3.0
1	A	241	GLY	3.0
1	A	83	VAL	3.0
1	A	142	ARG	3.0
1	A	167	PHE	3.0
1	A	131	ILE	3.0
1	A	136	TYR	3.0
1	A	3	GLU	2.9
1	A	246	ALA	2.9
1	A	152	THR	2.9
1	A	300	VAL	2.9
1	A	187	LEU	2.9
1	A	112	ILE	2.9
1	A	244	ILE	2.9
1	A	180	GLU	2.9
1	A	305	ALA	2.9
1	A	271	TYR	2.8
1	A	22	LEU	2.8
1	A	132	ARG	2.8
1	A	130	LEU	2.8
1	A	294	LYS	2.8
1	A	235	PHE	2.8
1	A	320	GLY	2.8

Continued from previous page...

Mol	Chain	Res	Type	RSRZ
1	A	70	LEU	2.8
1	A	249	LEU	2.8
1	A	280	ALA	2.8
1	A	230	TYR	2.8
1	A	192	VAL	2.8
1	A	258	LEU	2.8
1	A	251	ASP	2.8
1	A	331	ILE	2.7
1	A	28	VAL	2.7
1	A	204	ALA	2.7
1	A	8	TRP	2.7
1	A	281	LEU	2.7
1	A	299	ILE	2.7
1	A	353	PRO	2.7
1	A	6	ILE	2.7
1	A	218	ILE	2.7
1	A	154	ALA	2.7
1	A	69	ARG	2.7
1	A	46	VAL	2.6
1	A	36	LYS	2.6
1	A	49	LYS	2.6
1	A	301	ASP	2.6
1	A	307	SER	2.6
1	A	32	LEU	2.6
1	A	191	LEU	2.6
1	A	159	GLY	2.6
1	A	264	VAL	2.6
1	A	20	GLN	2.6
1	A	302	HIS	2.6
1	A	76	ASN	2.6
1	A	149	CYS	2.6
1	A	346	TYR	2.5
1	A	56	ALA	2.5
1	A	332	PRO	2.5
1	A	193	ILE	2.5
1	A	228	ILE	2.5
1	A	325	GLY	2.5
1	A	93	LEU	2.5
1	A	110	ILE	2.5
1	A	339	THR	2.4
1	A	73	ASN	2.4
1	A	147	ALA	2.4

Continued on next page...

Continued from previous page...

Mol	Chain	Res	Type	RSRZ
1	A	282	VAL	2.4
1	A	349	SER	2.4
1	A	297	VAL	2.4
1	A	255	TYR	2.4
1	A	75	LEU	2.3
1	A	324	THR	2.3
1	A	291	SER	2.3
1	A	4	LYS	2.3
1	A	327	TRP	2.3
1	A	225	VAL	2.3
1	A	45	TYR	2.3
1	A	292	TYR	2.3
1	A	14	PHE	2.3
1	A	71	PHE	2.3
1	A	127	ASN	2.3
1	A	317	GLU	2.3
1	A	148	SER	2.2
1	A	295	GLN	2.2
1	A	223	LEU	2.2
1	A	330	LEU	2.2
1	A	171	PRO	2.2
1	A	253	LYS	2.2
1	A	303	HIS	2.2
1	A	113	PHE	2.2
1	A	195	VAL	2.2
1	A	248	ASN	2.2
1	A	272	ASN	2.2
1	A	342	PHE	2.2
1	A	361	PRO	2.1
1	A	134	ALA	2.1
1	A	153	ALA	2.1
1	A	309	PHE	2.1
1	A	138	SER	2.1
1	A	269	ALA	2.1
1	A	261	VAL	2.1
1	A	7	LEU	2.1
1	A	245	GLY	2.1
1	A	42	THR	2.1
1	A	111	THR	2.1
1	A	277	LYS	2.1
1	A	345	SER	2.1
1	A	126	TRP	2.1

Continued on next page...

Continued from previous page...

Mol	Chain	Res	Type	RSRZ
1	A	265	ILE	2.1
1	A	304	THR	2.0
1	A	216	PRO	2.0
1	A	315	GLN	2.0
1	A	219	SER	2.0
1	A	226	GLY	2.0
1	A	287	ALA	2.0
1	A	318	GLU	2.0
1	A	106	ILE	2.0
1	A	170	LEU	2.0
1	A	150	SER	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 carbohydrates 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. LLDF column lists the quality of electron density of the group with respect to its neighbouring residues in protein, DNA or RNA chains. The B-factors column lists the minimum, median, 95th 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	LLDF	B-factors(Å^2)	Q<0.9
5	HW1	A	904	28/28	0.66	0.33	2.69	24,50,67,68	0
3	H4B	A	902	17/17	0.74	0.30	1.72	41,50,76,77	0
6	GOL	A	905	6/6	0.71	0.22	0.22	53,55,58,60	0
2	HEM	A	901	43/43	0.85	0.21	-0.33	19,25,40,45	0
4	CL	A	903	1/1	0.97	0.07	-4.30	30,30,30,30	0

6.5 Other polymers (i)

There are no such residues in this entry.