



Full wwPDB X-ray Structure Validation Report ⓘ

Mar 5, 2026 – 05:03 PM UTC

PDB ID : 4D3V / pdb_00004d3v
Title : Structure of Bacillus subtilis Nitric Oxide Synthase I218V in complex with N-{3-[(1S)-2-(3-{(Z)-[amino(thiophen-2-yl)methylidene]amino}phenoxy)-1-hydroxyethyl]phenyl}thiophene-2-carboximidamide
Authors : Holden, J.K.; Poulos, T.L.
Deposited on : 2014-10-23
Resolution : 1.88 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 ⓘ 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 ⓘ](#)) were used in the production of this report:

MolProbity	:	4-5-2 with Phenix2.0
Mogul	:	2022.3.0, CSD as543be (2022)
Xtriage (Phenix)	:	2.0
EDS	:	3.0
Buster-report	:	wwPDB partial adaption of 1.1.7 (2018)
Percentile statistics	:	20250101.v01 (using entries in the PDB archive January 1st 2025)
CCP4	:	9.0.010 (Gargrove)
Density-Fitness	:	1.0.12
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.49

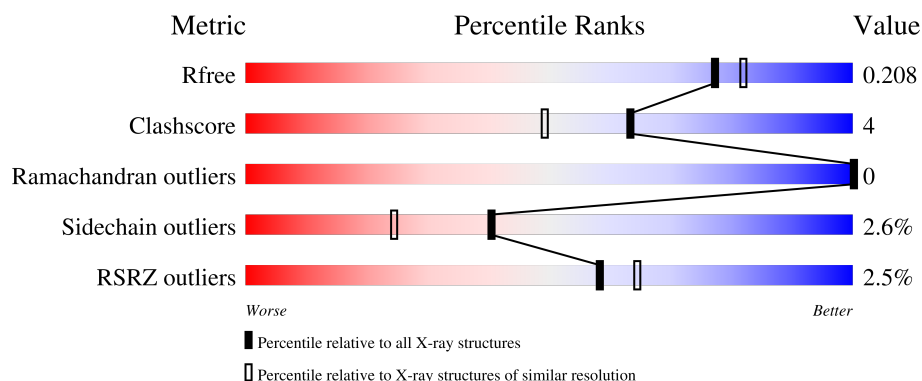
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

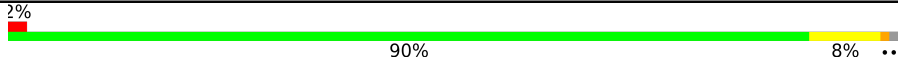
The reported resolution of this entry is 1.88 Å.

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	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
R_{free}	180053	1220 (1.88-1.88)
Clashscore	190562	1234 (1.88-1.88)
Ramachandran outliers	187476	1222 (1.88-1.88)
Sidechain outliers	187428	1222 (1.88-1.88)
RSRZ outliers	180081	1220 (1.88-1.88)

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 $\leq 5\%$. 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	366	

2 Entry composition [i](#)

There are 7 unique types of molecules in this entry. The entry contains 3318 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.

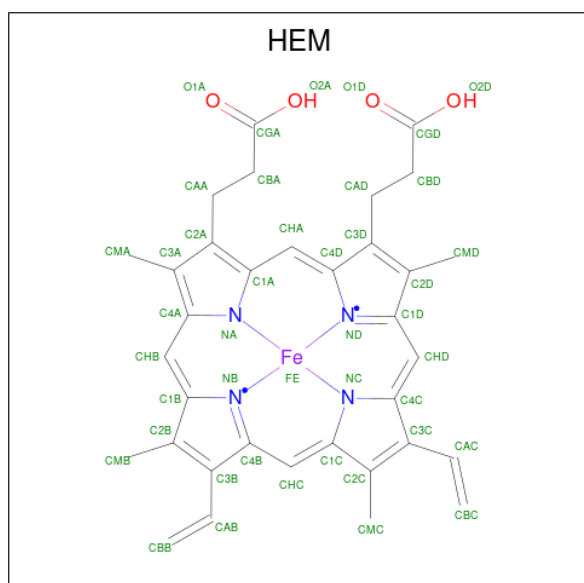
- Molecule 1 is a protein called NITRIC OXIDE SYNTHASE OXYGENASE.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	362	Total	C	N	O	S	0	0	0
			2940	1872	507	553	8			

There are 7 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-2	GLY	-	expression tag	UNP O34453
A	-1	SER	-	expression tag	UNP O34453
A	0	HIS	-	expression tag	UNP O34453
A	25	ALA	GLU	engineered mutation	UNP O34453
A	26	ALA	GLU	engineered mutation	UNP O34453
A	218	VAL	ILE	engineered mutation	UNP O34453
A	316	ALA	GLU	engineered mutation	UNP O34453

- Molecule 2 is PROTOPORPHYRIN IX CONTAINING FE (CCD ID: HEM) (formula: $C_{34}H_{32}FeN_4O_4$).

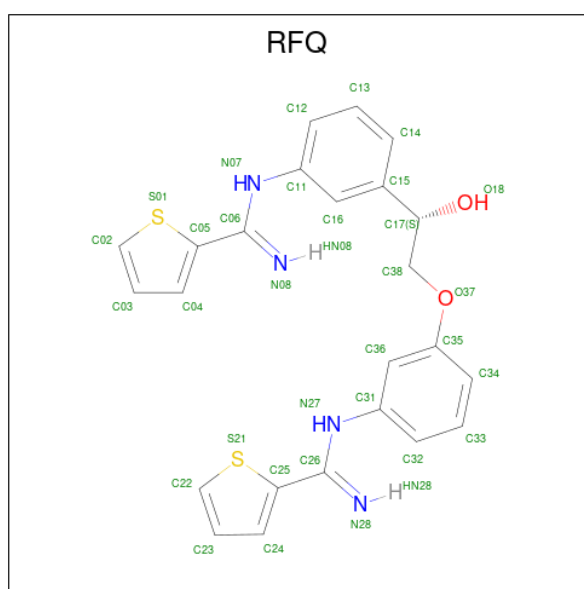


Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
2	A	1	Total	C	Fe	N	O	
			43	34	1	4	4	
							0	0

- Molecule 3 is CHLORIDE ION (CCD ID: CL) (formula: Cl).

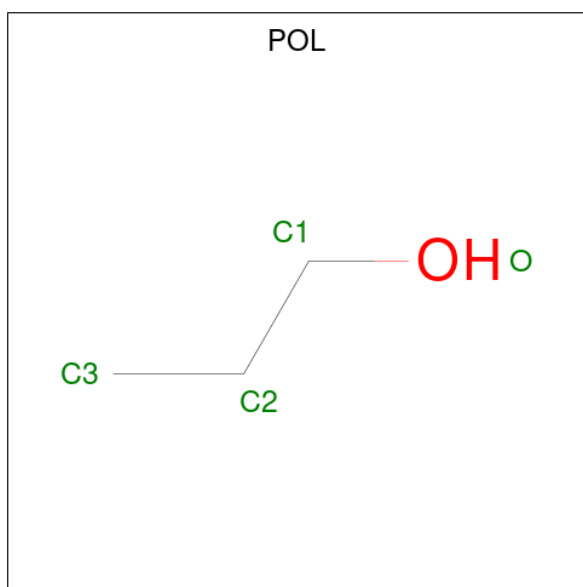
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
3	A	1	Total	Cl		
			1	1	0	0

- Molecule 4 is N-{3-[(1S)-2-(3-{(Z)-[amino(thiophen-2-yl)methylidene]amino}phenoxy)-1-hydroxyethyl]phenyl}thiophene-2-carboximidamide (CCD ID: RFQ) (formula: C₂₄H₂₂N₄O₂S₂).



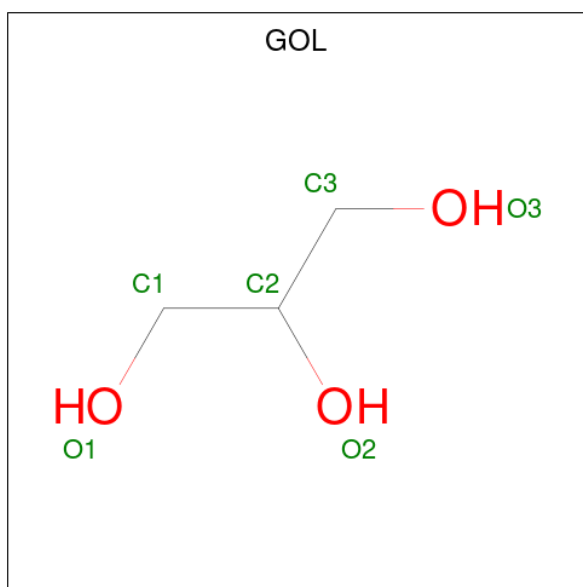
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
4	A	1	Total	C	N	O	S	
			32	24	4	2	2	
							0	0

- Molecule 5 is N-PROPANOL (CCD ID: POL) (formula: C₃H₈O).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
5	A	1	Total	C	O	0	0
			4	3	1		
5	A	1	Total	C	O	0	0
			4	3	1		
5	A	1	Total	C	O	0	0
			4	3	1		

- Molecule 6 is GLYCEROL (CCD ID: GOL) (formula: $C_3H_8O_3$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
6	A	1	Total	C	O	0	0
			6	3	3		

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Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
6	A	1	Total	C	O	0	0
			6	3	3		
6	A	1	Total	C	O	0	0
			6	3	3		

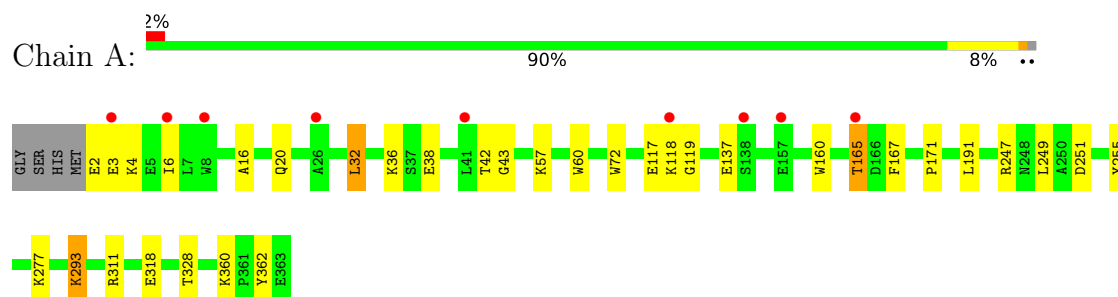
- Molecule 7 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
7	A	272	Total	O	0	0
			272	272		

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: 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 94.94Å 62.13Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	49.20 – 1.88 49.20 – 1.88	Depositor EDS
% Data completeness (in resolution range)	99.5 (49.20-1.88) 99.6 (49.20-1.88)	Depositor EDS
R_{merge}	0.07	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.79 (at 1.88Å)	Xtriage
Refinement program	PHENIX (PHENIX.REFINE)	Depositor
R, R_{free}	0.172 , 0.209 0.173 , 0.208	Depositor DCC
R_{free} test set	2006 reflections (5.09%)	wwPDB-VP
Wilson B-factor (Å ²)	30.2	Xtriage
Anisotropy	0.633	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.32 , 38.1	EDS
L-test for twinning ²	$\langle L \rangle = 0.49$, $\langle L^2 \rangle = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.97	EDS
Total number of atoms	3318	wwPDB-VP
Average B, all atoms (Å ²)	45.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 4.96% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical 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.

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: HEM, GOL, POL, CL, RFQ

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	0.42	0/3016	0.77	1/4087 (0.0%)

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	119	GLY	N-CA-C	-6.39	106.50	115.32

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	2940	0	2854	21	0
2	A	43	0	30	3	0
3	A	1	0	0	0	0
4	A	32	0	19	3	0
5	A	12	0	24	3	0
6	A	18	0	24	0	0
7	A	272	0	0	4	0
All	All	3318	0	2951	24	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 4.

All (24) 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:165:THR:HG23	1:A:167:PHE:H	1.55	0.71
1:A:16:ALA:O	1:A:20:GLN:HG2	1.99	0.63
1:A:247:ARG:NH1	1:A:251:ASP:OD2	2.31	0.63
1:A:165:THR:CG2	1:A:167:PHE:H	2.12	0.61
1:A:2:GLU:N	1:A:2:GLU:OE1	2.34	0.60
2:A:901:HEM:O2A	4:A:904:RFQ:H36	2.05	0.55
1:A:247:ARG:HB3	5:A:906:POL:H31	1.89	0.54
1:A:60:TRP:CE3	1:A:72:TRP:HA	2.44	0.52
1:A:277:LYS:NZ	7:A:2210:HOH:O	2.40	0.51
1:A:328:THR:O	4:A:904:RFQ:H23	2.13	0.48
1:A:57:LYS:NZ	7:A:2019:HOH:O	2.42	0.48
1:A:2:GLU:HB2	5:A:907:POL:H22	1.96	0.47
1:A:3:GLU:HG3	5:A:907:POL:H21	1.97	0.47
1:A:38:GLU:O	1:A:42:THR:OG1	2.28	0.46
2:A:901:HEM:HBB2	2:A:901:HEM:HHC	1.96	0.45
1:A:32:LEU:HD12	1:A:32:LEU:HA	1.79	0.45
2:A:901:HEM:O2A	4:A:904:RFQ:H38A	2.18	0.44
1:A:360:LYS:HD3	1:A:362:TYR:CZ	2.53	0.44
1:A:117:GLU:HG2	1:A:118:LYS:HG3	1.99	0.43
1:A:4:LYS:HE2	1:A:43:GLY:HA2	2.01	0.42
1:A:293:LYS:HE2	7:A:2215:HOH:O	2.20	0.42
1:A:311:ARG:HD3	7:A:2228:HOH:O	2.19	0.42
1:A:160:TRP:CE2	1:A:171:PRO:HD3	2.55	0.41
1:A:249:LEU:HA	1:A:255:TYR:HB2	2.02	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	Favoured	Allowed	Outliers	Percentiles	
1	A	360/366 (98%)	351 (98%)	9 (2%)	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	309/312 (99%)	301 (97%)	8 (3%)	40	24

All (8) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	6	ILE
1	A	32	LEU
1	A	36	LYS
1	A	137	GLU
1	A	165	THR
1	A	191	LEU
1	A	293	LYS
1	A	318	GLU

Sometimes 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 [i](#)

There are no oligosaccharides in this entry.

5.6 Ligand geometry [i](#)

Of 9 ligands modelled in this entry, 1 is monoatomic - leaving 8 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
6	GOL	A	909	-	5,5,5	0.34	0	5,5,5	0.37	0
5	POL	A	907	-	3,3,3	0.39	0	2,2,2	0.23	0
5	POL	A	906	-	3,3,3	0.39	0	2,2,2	0.21	0
6	GOL	A	910	-	5,5,5	0.41	0	5,5,5	0.59	0
4	RFQ	A	904	-	35,35,35	1.14	3 (8%)	41,47,47	1.41	7 (17%)
6	GOL	A	908	-	5,5,5	0.44	0	5,5,5	0.45	0
2	HEM	A	901	1	50,50,50	1.50	6 (12%)	67,82,82	1.68	16 (23%)
5	POL	A	905	-	3,3,3	0.35	0	2,2,2	0.35	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
6	GOL	A	909	-	-	3/4/4/4	-
5	POL	A	907	-	-	0/1/1/1	-
5	POL	A	906	-	-	1/1/1/1	-
6	GOL	A	910	-	-	3/4/4/4	-
4	RFQ	A	904	-	-	3/17/25/25	0/4/4/4
6	GOL	A	908	-	-	2/4/4/4	-
2	HEM	A	901	1	-	1/14/54/54	-
5	POL	A	905	-	-	0/1/1/1	-

All (9) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	901	HEM	FE-NB	5.31	2.11	1.94
2	A	901	HEM	FE-NC	4.40	2.09	1.95
2	A	901	HEM	C1B-NB	-3.03	1.35	1.40
4	A	904	RFQ	C25-C26	2.93	1.50	1.45
2	A	901	HEM	C3B-C4B	2.45	1.49	1.44
4	A	904	RFQ	C06-N07	-2.29	1.34	1.38
2	A	901	HEM	C4D-ND	-2.22	1.36	1.40
4	A	904	RFQ	C11-N07	-2.10	1.37	1.41
2	A	901	HEM	O2A-CGA	-2.03	1.24	1.30

All (23) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	A	901	HEM	CHC-C4B-NB	4.61	129.38	124.42
2	A	901	HEM	CHD-C1D-ND	4.03	128.76	124.42
4	A	904	RFQ	C06-C05-S01	-3.32	114.64	120.28
4	A	904	RFQ	C24-C25-C26	3.16	134.89	128.65
2	A	901	HEM	CHA-C4D-ND	3.10	128.20	124.37
2	A	901	HEM	C1A-CHA-C4D	-3.04	119.10	126.25
2	A	901	HEM	CHA-C4D-C3D	-2.99	119.72	125.23
2	A	901	HEM	C1B-NB-C4B	2.96	108.71	105.21
4	A	904	RFQ	C23-C22-S21	-2.93	105.85	113.02
2	A	901	HEM	CHD-C1D-C2D	-2.89	120.47	125.03
2	A	901	HEM	O2D-CGD-CBD	2.71	122.57	114.00
2	A	901	HEM	C3B-C4B-NB	-2.61	107.59	109.47
2	A	901	HEM	C4A-CHB-C1B	-2.51	120.34	126.25
4	A	904	RFQ	C38-O37-C35	2.40	123.13	117.85
4	A	904	RFQ	C22-S21-C25	2.38	96.61	92.33
2	A	901	HEM	C4C-NC-C1C	2.36	109.66	105.82
2	A	901	HEM	CHA-C1A-NA	2.35	128.13	123.86
2	A	901	HEM	CAD-C3D-C4D	2.34	128.77	124.70
4	A	904	RFQ	C04-C05-C06	2.21	133.02	128.65
2	A	901	HEM	C4C-CHD-C1D	-2.19	121.36	126.02
4	A	904	RFQ	C25-C26-N27	2.19	120.47	116.12
2	A	901	HEM	CHB-C1B-NB	2.19	127.08	124.37
2	A	901	HEM	CMD-C2D-C1D	2.11	128.32	125.03

There are no chirality outliers.

All (13) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
4	A	904	RFQ	O18-C17-C38-O37

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Mol	Chain	Res	Type	Atoms
6	A	908	GOL	C1-C2-C3-O3
6	A	909	GOL	O1-C1-C2-C3
6	A	908	GOL	O2-C2-C3-O3
6	A	909	GOL	O1-C1-C2-O2
6	A	910	GOL	O1-C1-C2-O2
5	A	906	POL	O-C1-C2-C3
4	A	904	RFQ	C15-C17-C38-O37
6	A	910	GOL	O1-C1-C2-C3
6	A	909	GOL	C1-C2-C3-O3
4	A	904	RFQ	C36-C31-N27-C26
6	A	910	GOL	O2-C2-C3-O3
2	A	901	HEM	CAD-CBD-CGD-O2D

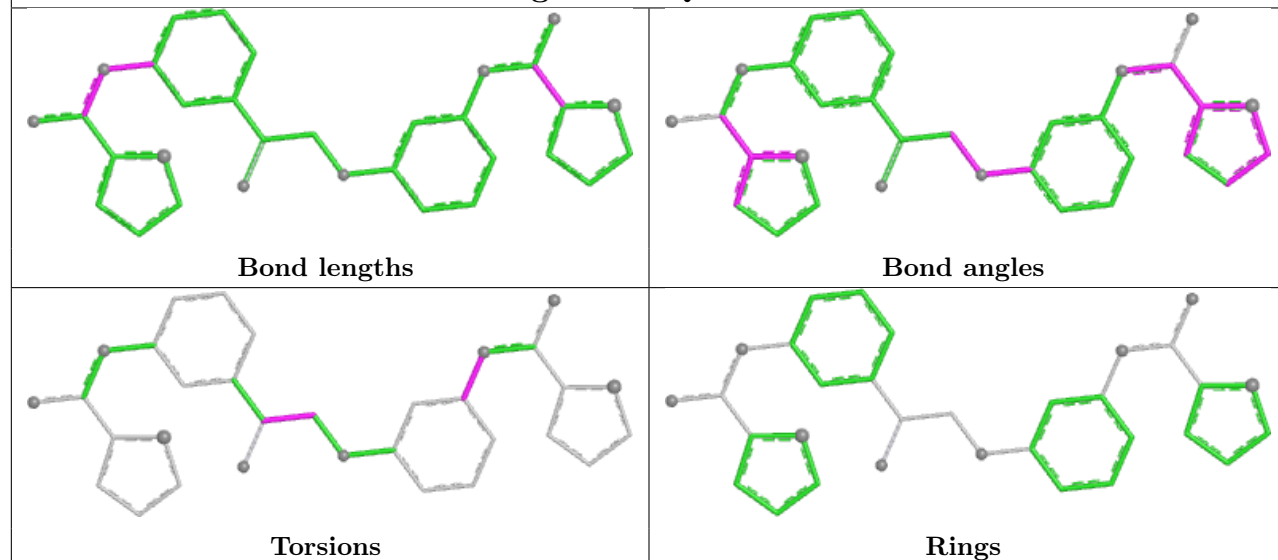
There are no ring outliers.

4 monomers are involved in 7 short contacts:

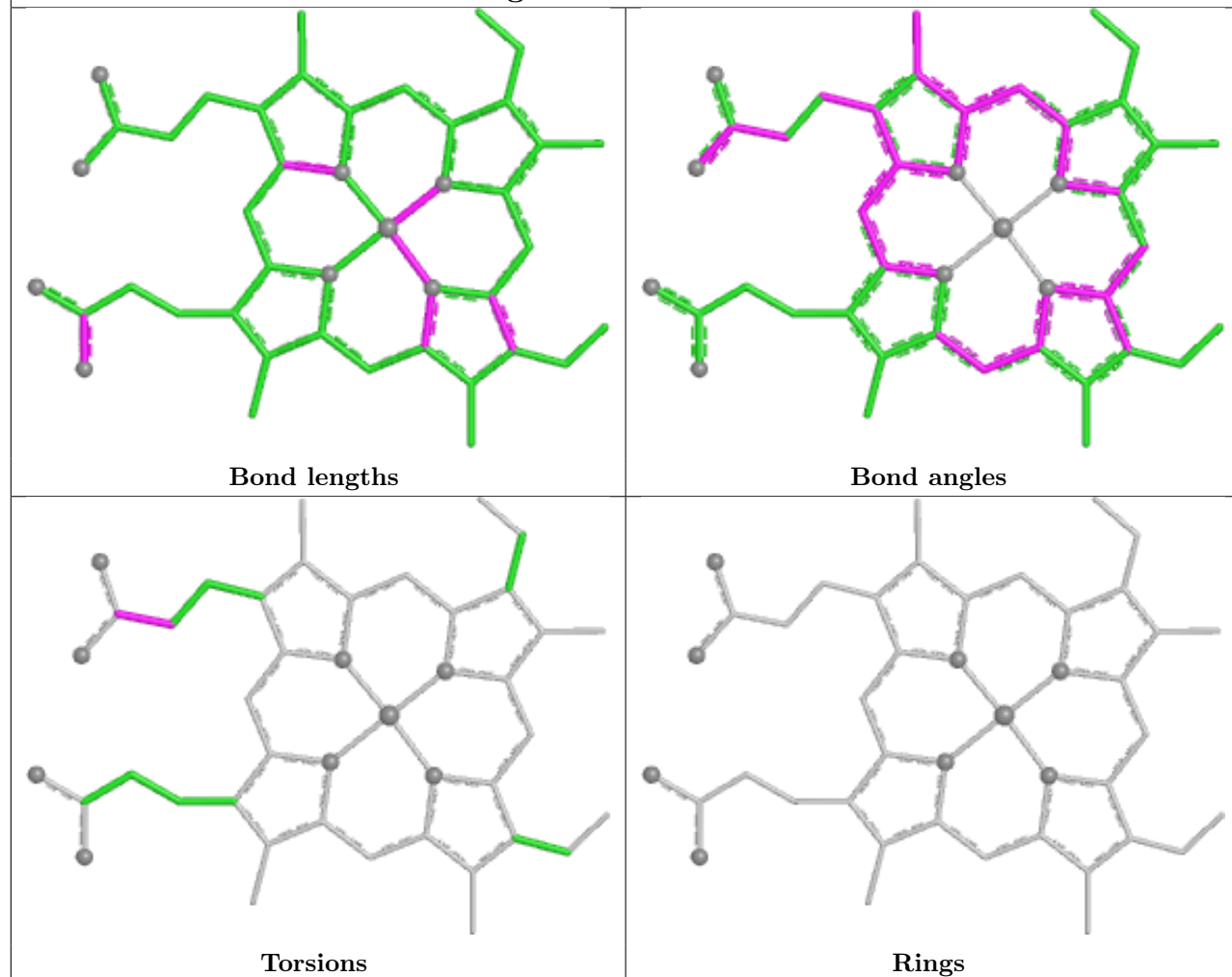
Mol	Chain	Res	Type	Clashes	Symm-Clashes
5	A	907	POL	2	0
5	A	906	POL	1	0
4	A	904	RFQ	3	0
2	A	901	HEM	3	0

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 than 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.

Ligand RFQ A 904



Ligand HEM A 901



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, 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	362/366 (98%)	0.07	9 (2%) 58 64	27, 40, 79, 105	0

All (9) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	8	TRP	3.0
1	A	41	LEU	2.9
1	A	6	ILE	2.6
1	A	165	THR	2.6
1	A	157	GLU	2.5
1	A	118	LYS	2.3
1	A	26	ALA	2.1
1	A	138	SER	2.1
1	A	3	GLU	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](#)

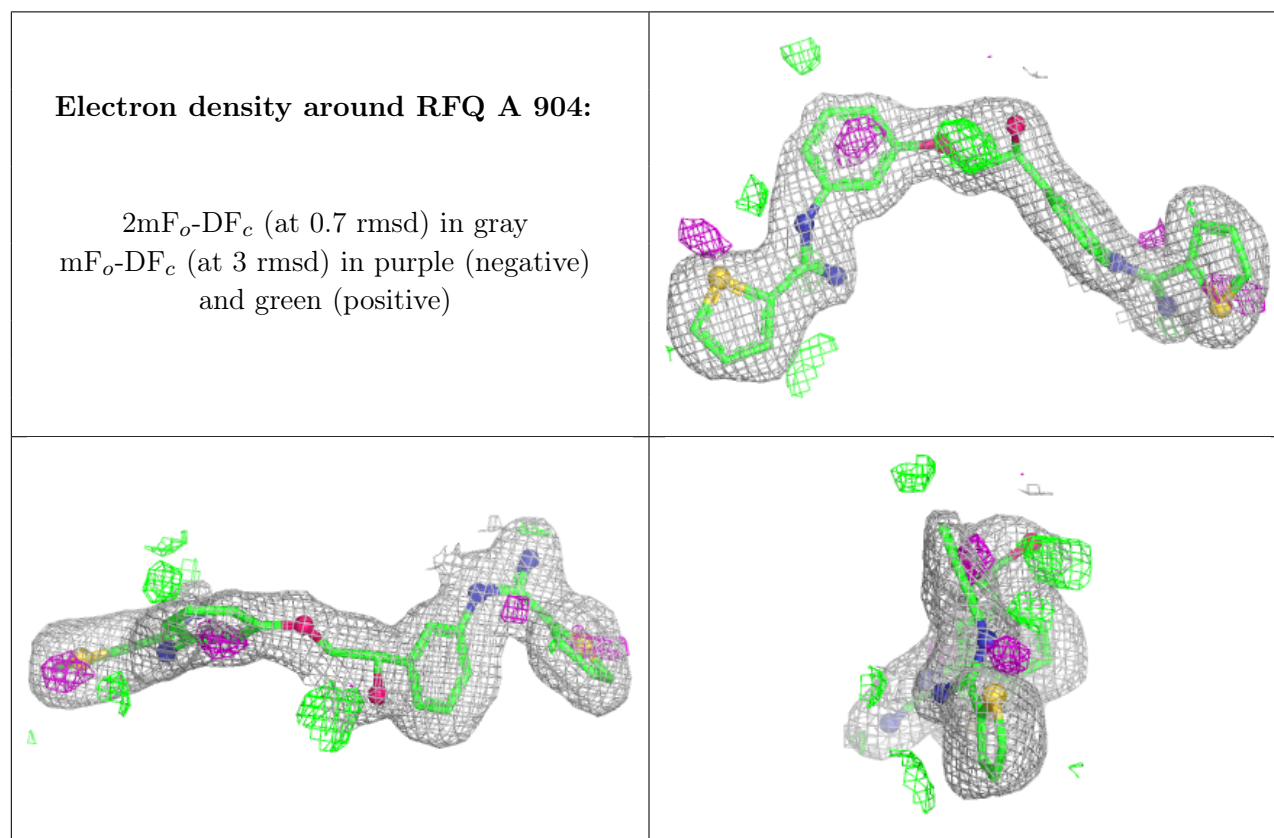
There are no oligosaccharides in this entry.

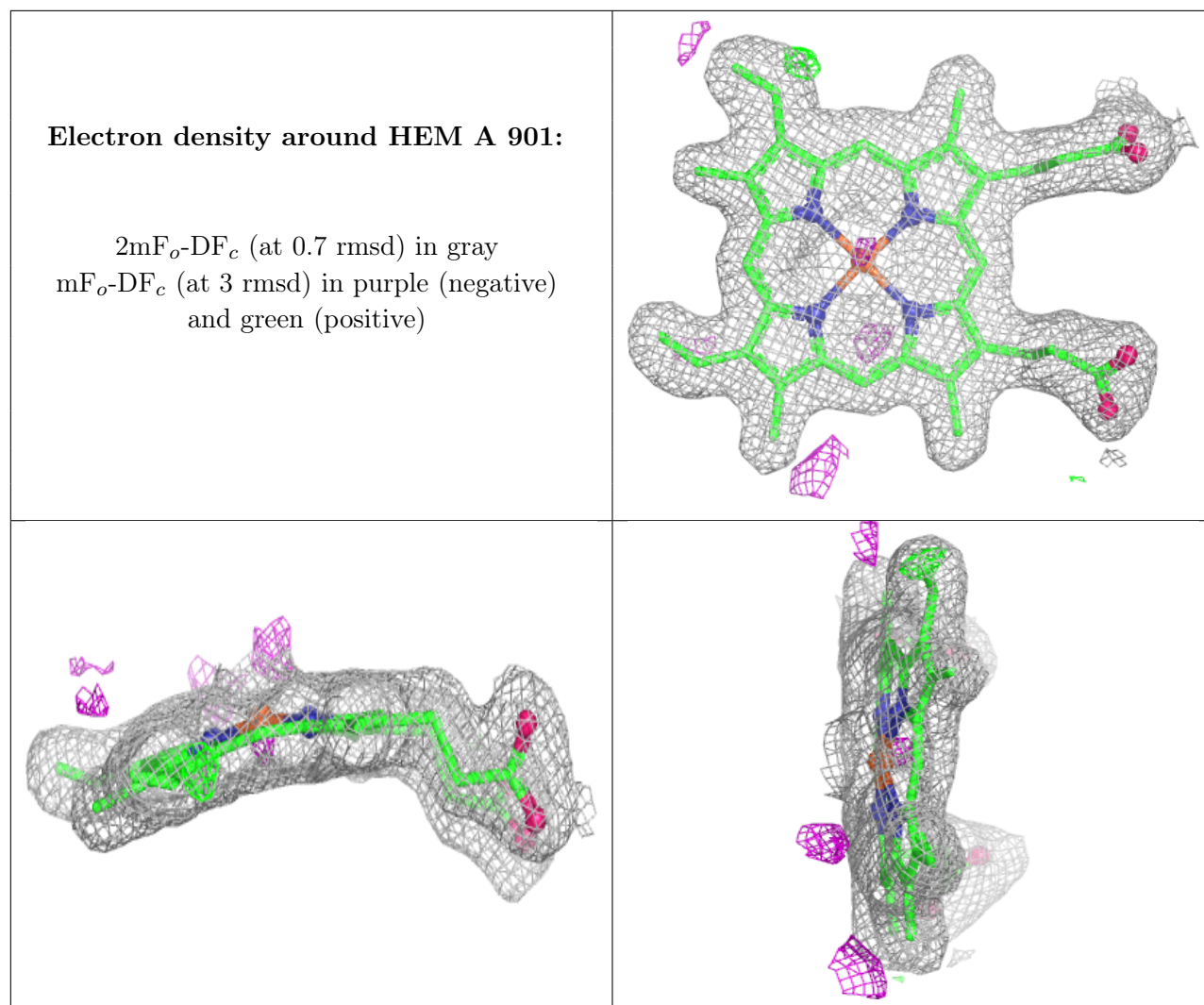
6.4 Ligands [i](#)

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, 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	B-factors($\text{\AA}^2$)	Q<0.9
5	POL	A	907	4/4	0.59	0.24	69,73,81,84	0
5	POL	A	906	4/4	0.79	0.19	38,42,44,47	0
6	GOL	A	909	6/6	0.81	0.17	64,69,72,79	0
6	GOL	A	910	6/6	0.81	0.22	47,56,65,66	0
6	GOL	A	908	6/6	0.84	0.14	47,58,63,66	0
5	POL	A	905	4/4	0.90	0.20	54,62,65,68	0
4	RFQ	A	904	32/32	0.94	0.10	20,41,59,75	0
2	HEM	A	901	43/43	0.98	0.07	25,29,38,50	0
3	CL	A	902	1/1	0.99	0.06	37,37,37,37	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 ⓘ

There are no such residues in this entry.