



Full wwPDB X-ray Structure Validation Report ⓘ

Mar 6, 2026 – 07:56 PM UTC

PDB ID : 8FGE / pdb_00008fge
Title : Structure of rat neuronal nitric oxide synthase R349A mutant heme domain in complex with 4-(difluoromethyl)-6-(5-(2-(dimethylamino)ethyl)-2,3-difluorophenethyl)pyridin-2-amine dihydrochloride
Authors : Li, H.; Poulos, T.L.
Deposited on : 2022-12-12
Resolution : 1.89 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

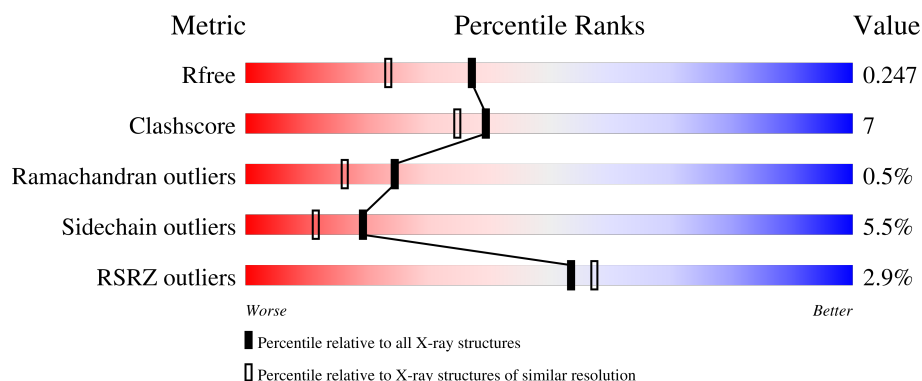
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 1.89 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	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
R_{free}	180053	7789 (1.90-1.90)
Clashscore	190562	8410 (1.90-1.90)
Ramachandran outliers	187476	8333 (1.90-1.90)
Sidechain outliers	187428	8333 (1.90-1.90)
RSRZ outliers	180081	7790 (1.90-1.90)

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	422	 3% 77% 19% ..

i

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.

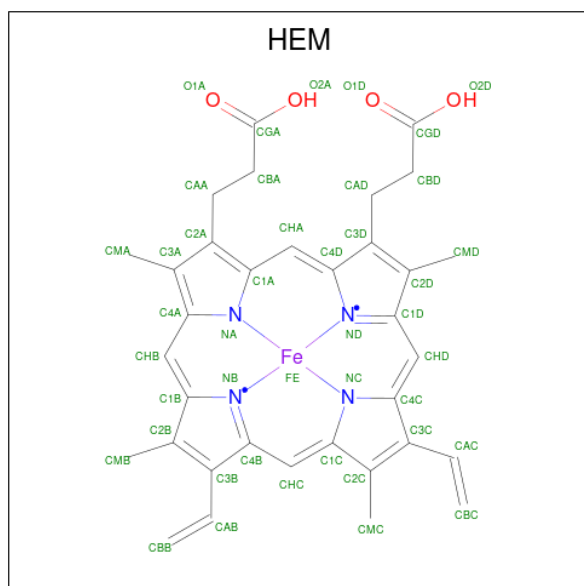
- Molecule 1 is a protein called Nitric oxide synthase, brain.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	411	Total	C	N	O	S	0	1	0
			3343	2140	571	611	21			

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	349	ALA	ARG	engineered mutation	UNP P29476

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



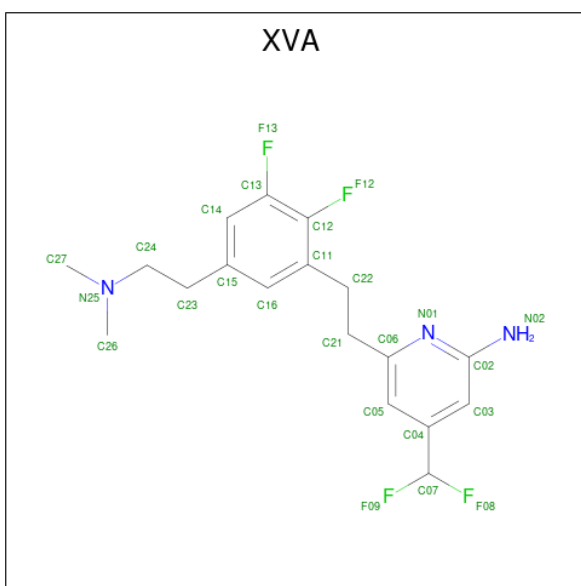
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
2	A	1	Total 43	C 34	Fe 1	N 4	O 4	0	0

- Molecule 3 is 5,6,7,8-TETRAHYDROBIOPTERIN (CCD ID: H4B) (formula: $C_9H_{15}N_5O_3$).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
3	A	1	Total	C	N	O	0	0
			17	9	5	3		

- Molecule 4 is 4-(difluoromethyl)-6-(2-{5-[2-(dimethylamino)ethyl]-2,3-difluorophenyl}ethyl)pyridin-2-amine (CCD ID: XVA) (formula: $C_{18}H_{21}F_4N_3$) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
4	A	1	Total	C	F	N	0	0
			25	18	4	3		

- Molecule 5 is ACETATE ION (CCD ID: ACT) (formula: $C_2H_3O_2$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
5	A	1	Total	C	O	0	0
			4	2	2		

- Molecule 6 is ZINC ION (CCD ID: ZN) (formula: Zn).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
6	A	1	Total	Zn	0	0
			1	1		

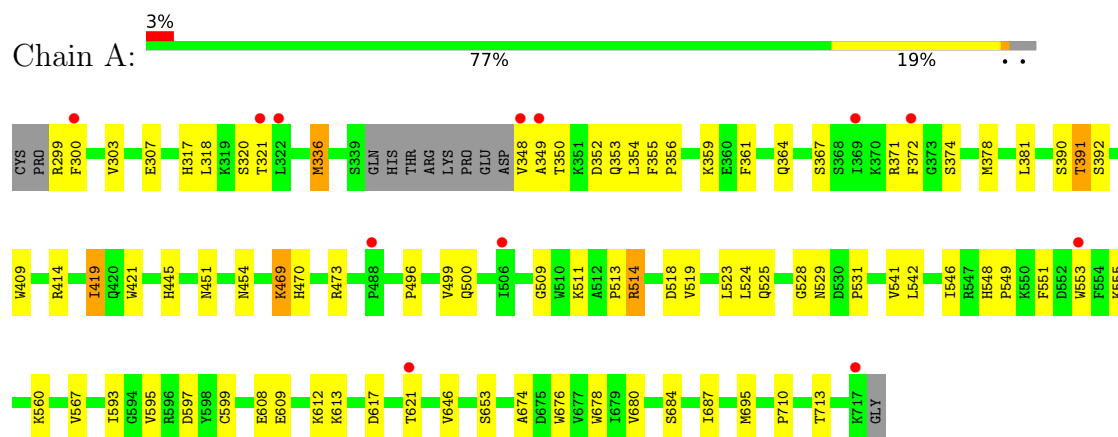
- Molecule 7 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
7	A	83	Total	O	0	0
			83	83		

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, brain



4 Data and refinement statistics

Property	Value	Source
Space group	C 2 2 21	Depositor
Cell constants a, b, c, α , β , γ	48.95Å 113.85Å 163.63Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	46.73 – 1.89 46.73 – 1.89	Depositor EDS
% Data completeness (in resolution range)	97.4 (46.73-1.89) 98.5 (46.73-1.89)	Depositor EDS
R_{merge}	0.13	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.49 (at 1.90Å)	Xtriage
Refinement program	PHENIX Jan 10, 2022	Depositor
R, R_{free}	0.209 , 0.248 0.214 , 0.247	Depositor DCC
R_{free} test set	1880 reflections (4.34%)	wwPDB-VP
Wilson B-factor (Å ²)	37.6	Xtriage
Anisotropy	1.145	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.34 , 53.8	EDS
L-test for twinning ²	$\langle L \rangle = 0.49$, $\langle L^2 \rangle = 0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.97	EDS
Total number of atoms	3516	wwPDB-VP
Average B, all atoms (Å ²)	75.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.39% 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: ZN, H4B, ACT, HEM, XVA

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	0.28	0/3439	0.48	0/4665

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	A	3343	0	3258	43	0
2	A	43	0	30	4	0
3	A	17	0	15	1	0
4	A	25	0	0	1	0
5	A	4	0	3	1	0
6	A	1	0	0	0	0
7	A	83	0	0	1	0
All	All	3516	0	3306	45	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 7.

All (45) 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:299:ARG:HG3	1:A:318:LEU:HD21	1.76	0.66
2:A:801:HEM:HMC2	2:A:801:HEM:HBC2	1.81	0.62
1:A:359:LYS:HG3	1:A:381:LEU:HD11	1.86	0.58
1:A:525:GLN:HE21	1:A:528:GLY:HA2	1.70	0.56
1:A:678:TRP:CE3	2:A:801:HEM:HBA2	2.44	0.53
1:A:519:VAL:HG21	1:A:541:VAL:HG11	1.90	0.52
1:A:473:ARG:NH2	1:A:710:PRO:HD3	2.24	0.52
1:A:336:MET:HG3	3:A:802:H4B:H9	1.91	0.51
1:A:542:LEU:HD21	1:A:646:VAL:HG22	1.93	0.50
1:A:551:PHE:HB3	1:A:553:TRP:CE2	2.47	0.50
1:A:348:VAL:HG22	1:A:349:ALA:H	1.77	0.49
1:A:525:GLN:HG3	1:A:529:ASN:O	2.12	0.49
1:A:317:HIS:O	1:A:320:SER:HB3	2.13	0.49
1:A:361:PHE:O	1:A:364:GLN:HG2	2.13	0.49
1:A:553:TRP:CE3	1:A:613:LYS:HD3	2.49	0.48
2:A:801:HEM:HBB2	2:A:801:HEM:HHC	1.96	0.47
1:A:419:ILE:HG22	5:A:804:ACT:H1	1.95	0.47
1:A:567:VAL:HG21	4:A:803:XVA:C12	2.45	0.47
1:A:367:SER:HA	1:A:372:PHE:HB2	1.97	0.47
1:A:593:ILE:HA	1:A:597:ASP:HB2	1.97	0.46
1:A:674:ALA:HB3	1:A:695:MET:HB3	1.98	0.46
1:A:470:HIS:O	7:A:901:HOH:O	2.20	0.45
1:A:299:ARG:NE	1:A:299:ARG:HA	2.31	0.45
1:A:676:TRP:CE2	1:A:680:VAL:HG21	2.52	0.45
1:A:409:TRP:CE3	1:A:421:TRP:HA	2.51	0.44
1:A:546:ILE:HG12	1:A:560:LYS:HA	2.00	0.44
1:A:676:TRP:CZ2	1:A:680:VAL:HG21	2.53	0.44
1:A:496:PRO:HA	1:A:499:VAL:HG23	1.99	0.44
1:A:374:SER:O	1:A:378:MET:HG2	2.18	0.44
1:A:524:LEU:O	1:A:531:PRO:HA	2.17	0.44
1:A:451:ASN:HB3	1:A:454:ASN:O	2.18	0.43
1:A:514:ARG:HE	1:A:514:ARG:HB2	1.69	0.43
1:A:445:HIS:CD2	1:A:445:HIS:C	2.97	0.43
1:A:612:LYS:HE2	1:A:612:LYS:HB3	1.84	0.43
1:A:548:HIS:CG	1:A:549:PRO:HD2	2.54	0.43
1:A:350:THR:N	1:A:353:GLN:OE1	2.46	0.42
1:A:414:ARG:HD3	1:A:678:TRP:CD2	2.55	0.42
1:A:684:SER:HB3	1:A:687:ILE:HG12	2.02	0.42
1:A:355:PHE:N	1:A:356:PRO:HD2	2.35	0.41
1:A:469:LYS:HD2	1:A:469:LYS:HA	1.81	0.41
1:A:513:PRO:HG2	1:A:518:ASP:CG	2.46	0.41
1:A:678:TRP:HE3	2:A:801:HEM:HBA2	1.85	0.41

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:509:GLY:O	1:A:511:LYS:HE3	2.21	0.40
1:A:391:THR:O	1:A:392:SER:OG	2.32	0.40
1:A:595:VAL:O	1:A:599:CYS:HB2	2.22	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	408/422 (97%)	389 (95%)	17 (4%)	2 (0%)	24 16

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	514	ARG
1	A	617	ASP

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	A	367/376 (98%)	347 (95%)	20 (5%)	20 12

All (20) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	300	PHE
1	A	303	VAL
1	A	307	GLU
1	A	321	THR
1	A	336	MET
1	A	352	ASP
1	A	354	LEU
1	A	371	ARG
1	A	390	SER
1	A	391	THR
1	A	419	ILE
1	A	469	LYS
1	A	500	GLN
1	A	523	LEU
1	A	555	LYS
1	A	608	GLU
1	A	609	GLU
1	A	621	THR
1	A	653	SER
1	A	713	THR

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

Mol	Chain	Res	Type
1	A	500	GLN
1	A	535	GLN
1	A	642	GLN
1	A	652	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 oligosaccharides 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
5	ACT	A	804	-	3,3,3	0.81	0	3,3,3	0.75	0
4	XVA	A	803	-	25,26,26	0.35	0	33,36,36	1.88	7 (21%)
2	HEM	A	801	1	50,50,50	1.84	9 (18%)	67,82,82	1.34	10 (14%)
3	H4B	A	802	-	17,18,18	0.82	1 (5%)	14,26,26	1.73	4 (28%)

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	XVA	A	803	-	-	4/14/14/14	0/2/2/2
2	HEM	A	801	1	-	5/14/54/54	-
3	H4B	A	802	-	-	0/8/17/17	0/2/2/2

All (10) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	801	HEM	FE-NC	6.08	2.15	1.95
2	A	801	HEM	FE-NA	5.42	2.13	1.95
2	A	801	HEM	FE-NB	4.79	2.09	1.94
2	A	801	HEM	FE-ND	3.70	2.06	1.94
2	A	801	HEM	CAC-C3C	2.84	1.55	1.47
2	A	801	HEM	CAB-C3B	2.78	1.54	1.47
2	A	801	HEM	CMC-C2C	2.33	1.55	1.50
2	A	801	HEM	CMB-C2B	2.29	1.55	1.50
2	A	801	HEM	CMA-C3A	2.23	1.55	1.50
3	A	802	H4B	C4-N3	-2.02	1.35	1.38

All (21) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
4	A	803	XVA	C02-N01-C06	5.08	121.87	118.07
4	A	803	XVA	C22-C11-C12	-4.06	117.47	120.73
4	A	803	XVA	F09-C07-C04	-3.90	106.36	110.71
3	A	802	H4B	C2-N1-C8A	3.68	119.86	113.36
4	A	803	XVA	C21-C06-N01	3.28	121.04	116.06
2	A	801	HEM	CBD-CAD-C3D	3.23	121.46	112.53
2	A	801	HEM	C2A-C1A-NA	-3.03	106.79	110.15
3	A	802	H4B	O4-C4-C4A	-2.93	120.19	127.26
4	A	803	XVA	C22-C21-C06	-2.79	106.80	113.01
2	A	801	HEM	C4A-NA-C1A	2.66	110.16	105.82
4	A	803	XVA	C05-C06-N01	-2.58	119.79	122.73
3	A	802	H4B	C4A-C4-N3	2.54	119.11	112.13
3	A	802	H4B	C2-N3-C4	-2.43	120.70	125.11
2	A	801	HEM	C3B-C4B-NB	-2.33	107.79	109.47
2	A	801	HEM	CAD-CBD-CGD	-2.24	107.72	113.67
2	A	801	HEM	C3A-C4A-NA	-2.22	106.59	110.14
2	A	801	HEM	O2D-CGD-CBD	2.19	120.94	114.00
4	A	803	XVA	N02-C02-N01	2.18	120.10	116.59
2	A	801	HEM	CAA-CBA-CGA	-2.14	107.98	113.67
2	A	801	HEM	C1B-NB-C4B	2.11	107.71	105.21
2	A	801	HEM	C3B-C2B-C1B	2.03	107.93	106.41

There are no chirality outliers.

All (9) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	A	801	HEM	C1A-C2A-CAA-CBA
4	A	803	XVA	C06-C21-C22-C11
4	A	803	XVA	C15-C23-C24-N25
2	A	801	HEM	C2A-CAA-CBA-CGA
2	A	801	HEM	C3A-C2A-CAA-CBA
4	A	803	XVA	C03-C04-C07-F09
2	A	801	HEM	CAD-CBD-CGD-O1D
2	A	801	HEM	CAD-CBD-CGD-O2D
4	A	803	XVA	C05-C04-C07-F09

There are no ring outliers.

4 monomers are involved in 7 short contacts:

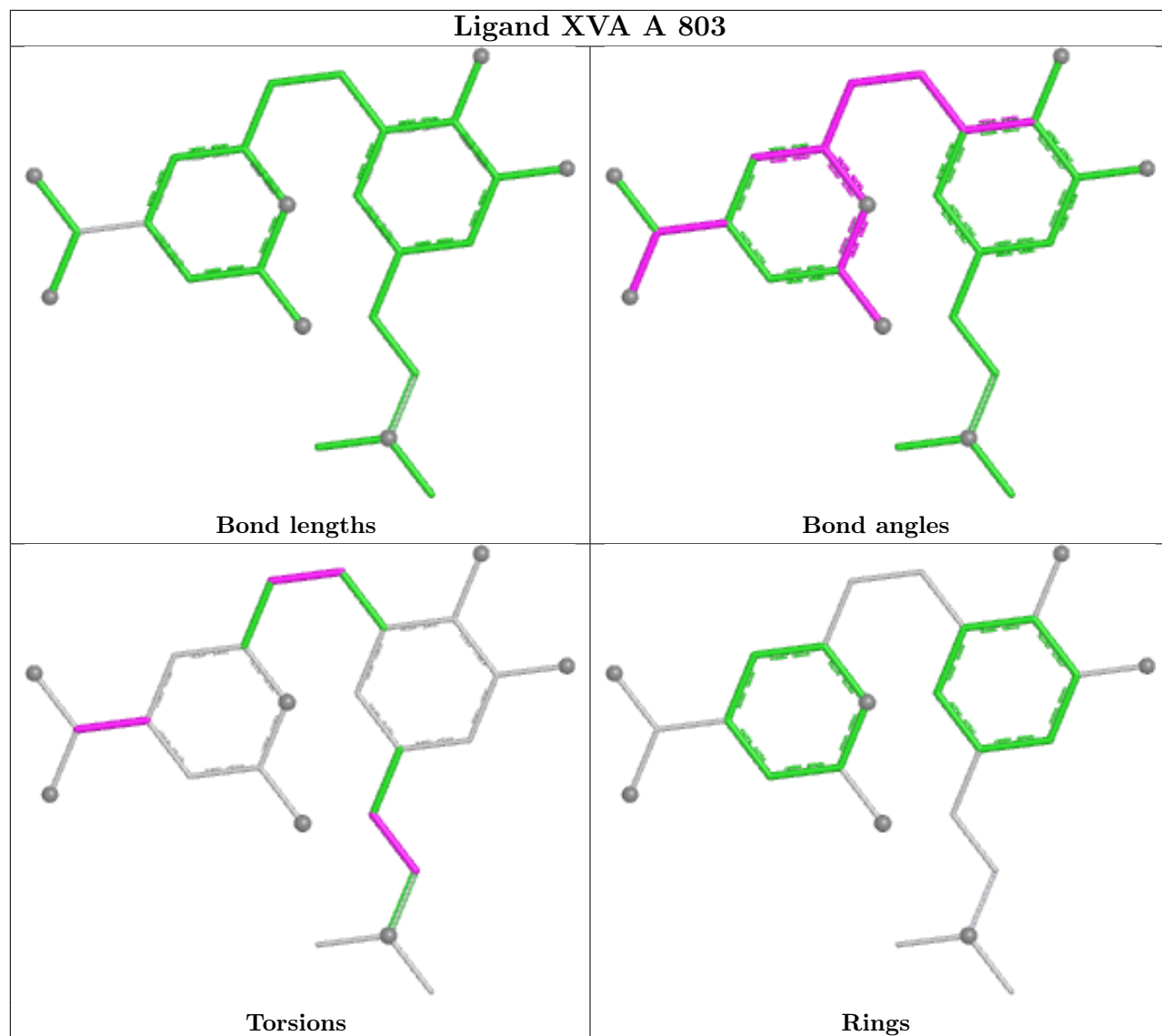
Mol	Chain	Res	Type	Clashes	Symm-Clashes
5	A	804	ACT	1	0
4	A	803	XVA	1	0

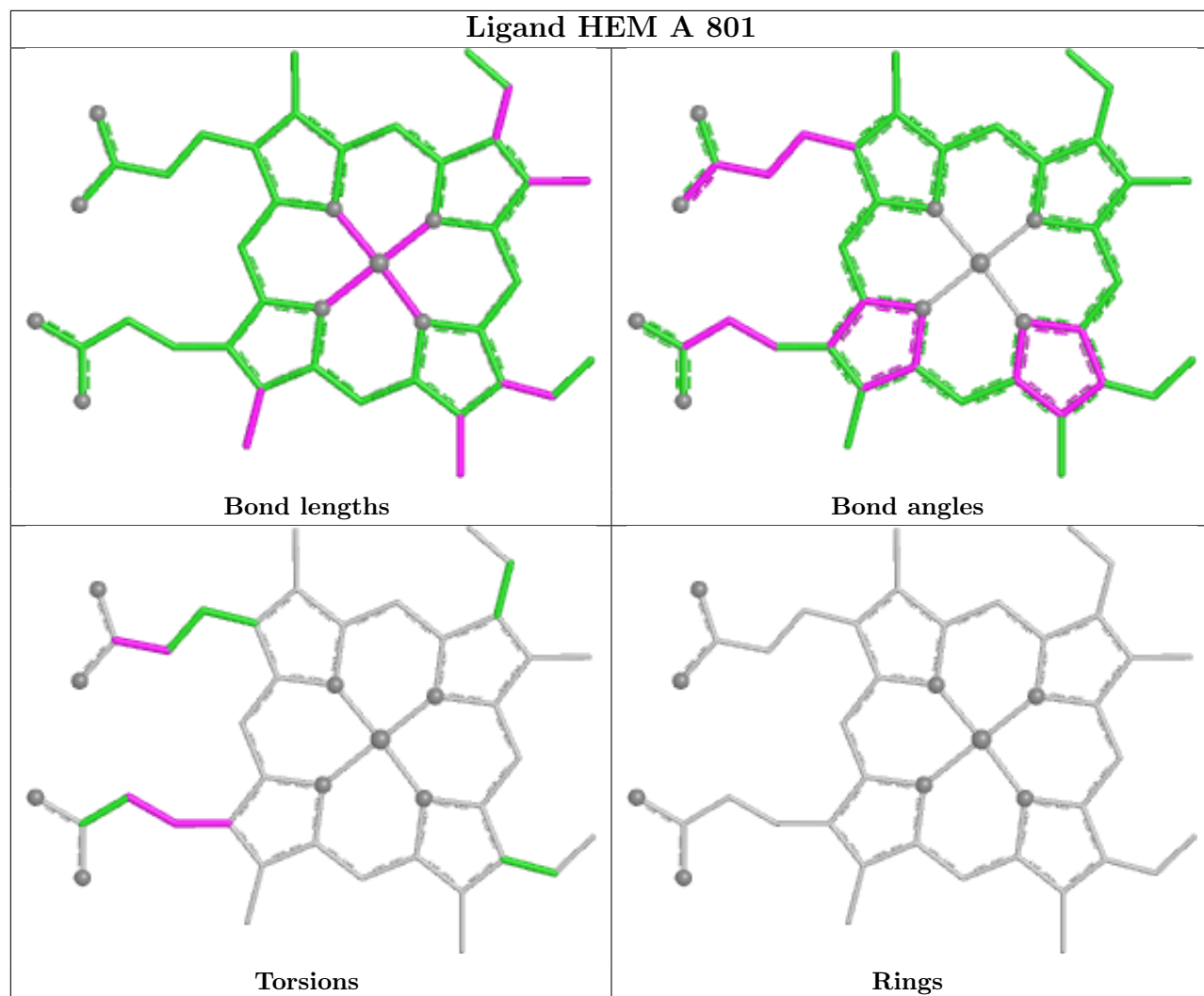
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Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	801	HEM	4	0
3	A	802	H4B	1	0

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.





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	411/422 (97%)	0.50	12 (2%) 53 57	38, 71, 128, 160	1 (0%)

All (12) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	300	PHE	3.5
1	A	349	ALA	3.1
1	A	348	VAL	2.9
1	A	369	ILE	2.8
1	A	717	LYS	2.6
1	A	372	PHE	2.6
1	A	321	THR	2.4
1	A	322	LEU	2.3
1	A	553	TRP	2.2
1	A	621	THR	2.2
1	A	506	ILE	2.0
1	A	488	PRO	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.

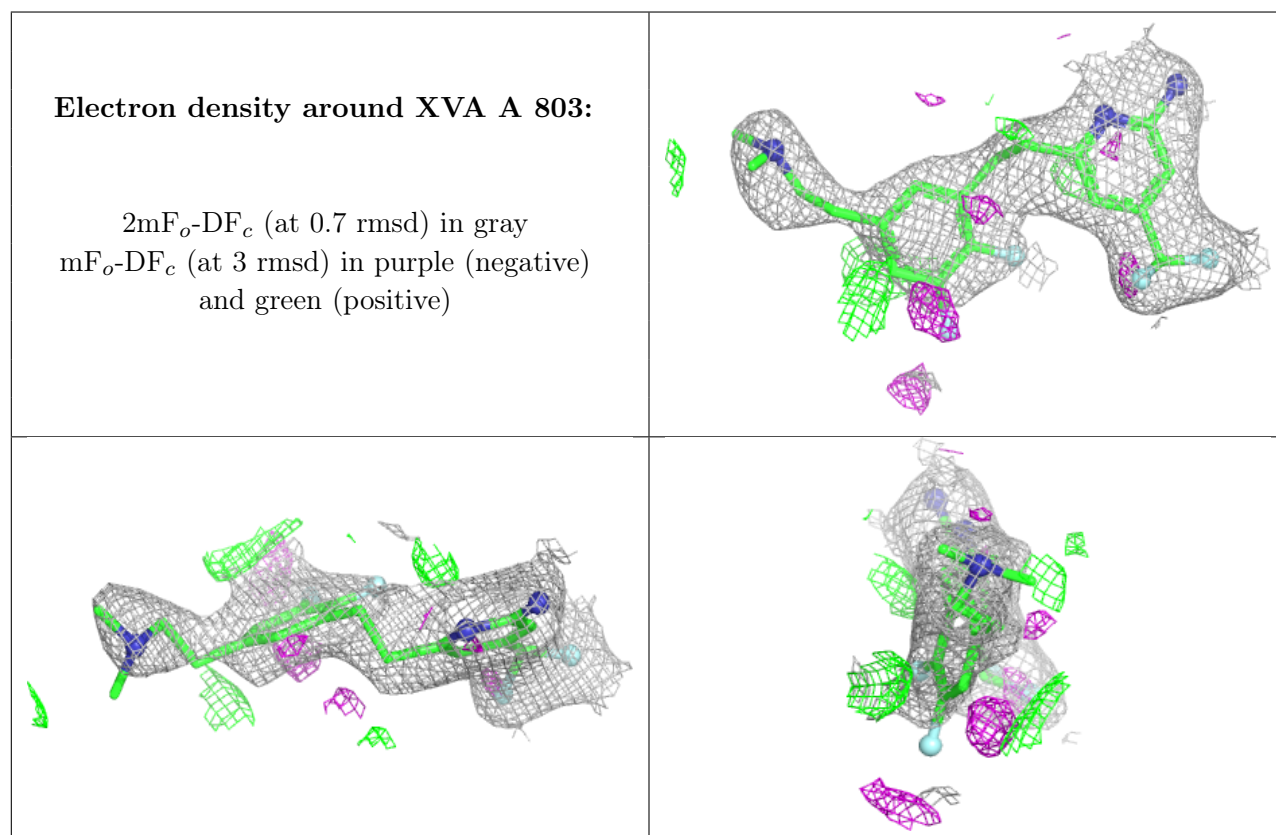
6.4 Ligands [i](#)

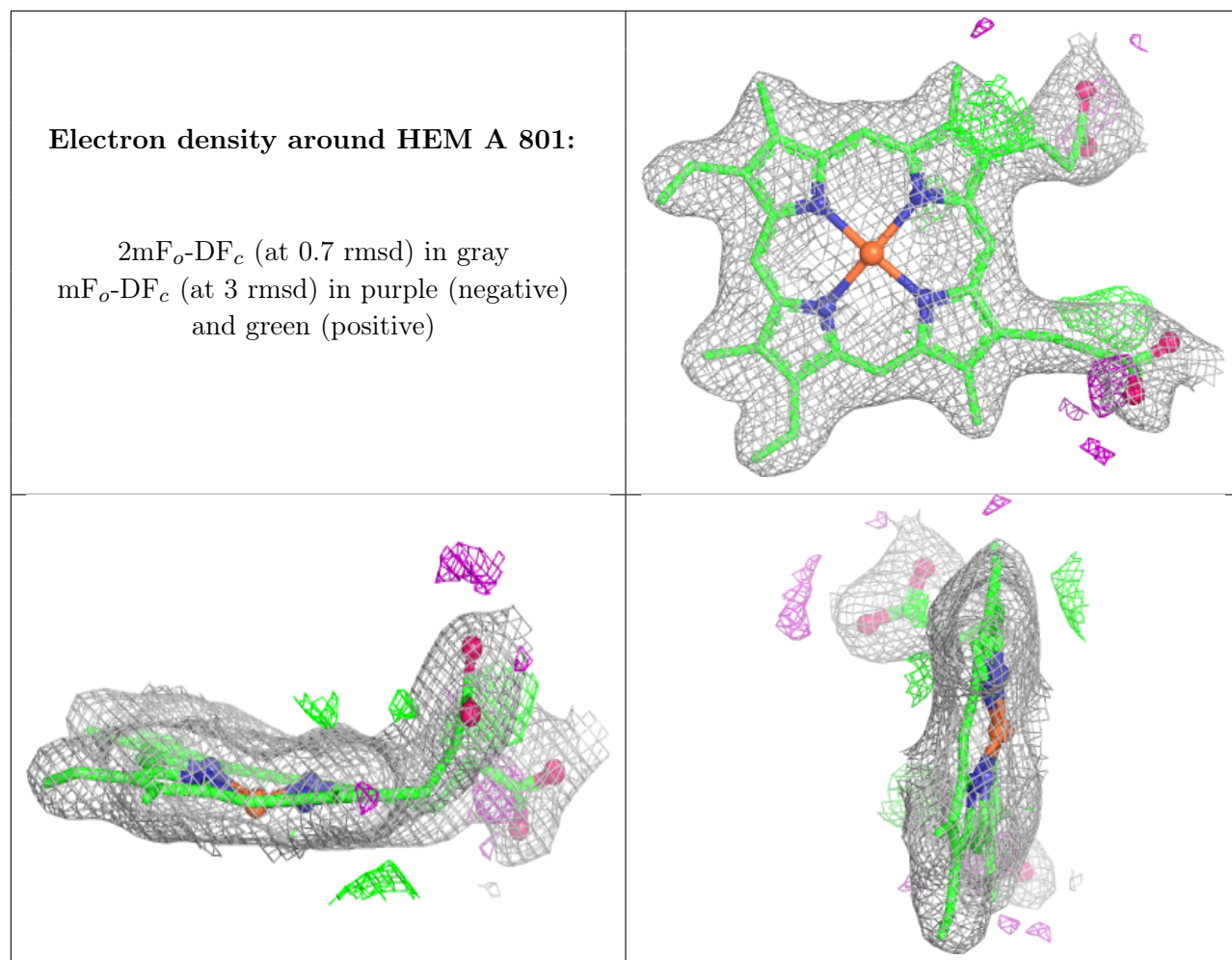
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum,

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(Å ²)	Q<0.9
4	XVA	A	803	25/25	0.88	0.15	46,80,110,121	0
5	ACT	A	804	4/4	0.89	0.20	111,113,113,113	0
3	H4B	A	802	17/17	0.95	0.07	40,45,48,50	0
2	HEM	A	801	43/43	0.97	0.08	33,47,59,62	0
6	ZN	A	805	1/1	1.00	0.05	48,48,48,48	1

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.