W O R L D W I D E

wwPDB

PROTEIN DATA BANK

wwPDB X-ray Structure Validation Summary Report (i)

Dec 15, 2024 – 06:29 AM EST

PDB ID	:	2BCN
Title	:	Solvent isotope effects on interfacial protein electron transfer between cytochrome c and cytochrome c peroxidase
Authors	:	Kang, S.A.; Crane, B.R.
Deposited on	:	2005-10-19
Resolution	:	1.70 Å(reported)

The following versions of software and data (see references (i)) were used in the production of this report:

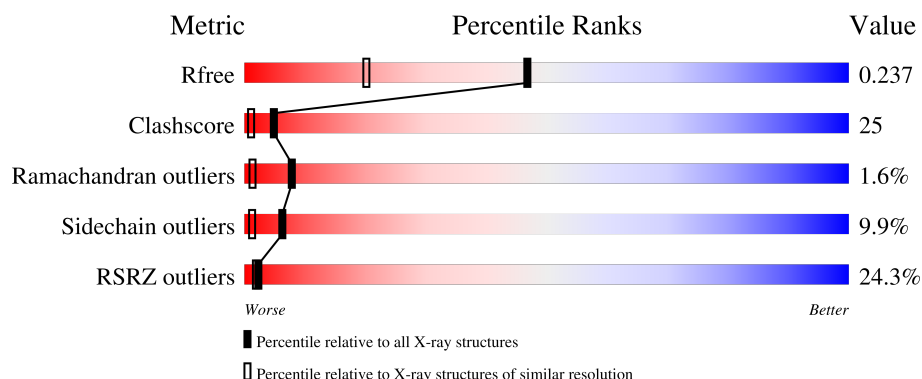|		
MolProbity	:	4.02b-467
Mogul	:	2022.3.0, CSD as543be (2022)
Xtriage (Phenix)	:	1.21
EDS	:	3.0
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20231227.v01 (using entries in the PDB archive December 27th 2023)
CCP4	:	9.0.004 (Gargrove)
Density-Fitness	:	1.0.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.40

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 1.70 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		0.237
Clashscore		25
Ramachandran outliers		1.6%
Sidechain outliers		9.9%
RSRZ outliers		24.3%

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$_{free}$	164625	5161 (1.70-1.70)
Clashscore	180529	5671 (1.70-1.70)
Ramachandran outliers	177936	5594 (1.70-1.70)
Sidechain outliers	177891	5594 (1.70-1.70)
RSRZ outliers	164620	5159 (1.70-1.70)

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%

Mol	Chain	Length	Quality of chain
1	A	296	12% 67% 20% 8% · ·
1	C	296	16% 68% 21% 8% ··
2	B	108	80% 36% 32% 27% 5%

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:

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Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
3	ZNH	A	295	X	-	-	-
3	ZNH	C	295	X	-	-	-

2 Entry composition ⓘ

There are 5 unique types of molecules in this entry. The entry contains 6265 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 cytochrome c peroxidase.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	294	Total 2371	C 1514	N 395	O 456	S 6	0	0	0
1	C	294	Total 2371	C 1514	N 395	O 456	S 6	0	0	0

There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-1	MET	-	cloning artifact	GB 171177
A	0	ILE	-	cloning artifact	GB 171177
C	-1	MET	-	cloning artifact	GB 171177
C	0	ILE	-	cloning artifact	GB 171177

- Molecule 2 is a protein called Cytochrome c iso-1.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	B	108	Total 847	C 534	N 151	O 158	S 4	0	0	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	107	SER	CYS	engineered mutation	UNP P00044

- Molecule 3 is PROTOPORPHYRIN IX CONTAINING ZN (three-letter code: ZNH) (formula: $C_{34}H_{32}N_4O_4Zn$).

ZNH

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

- Molecule 4 is HEME C (three-letter code: HEC) (formula: $C_{34}H_{34}FeN_4O_4$).

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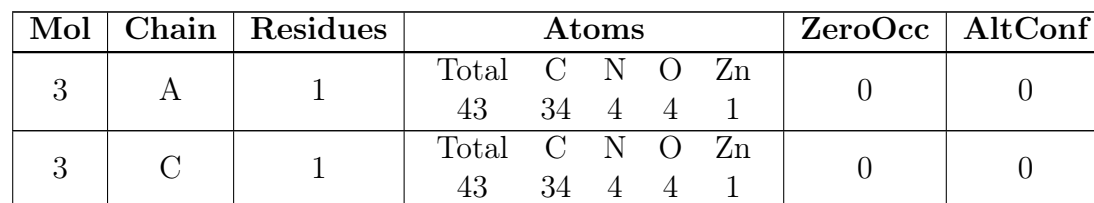
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
4	B	1	Total	C	Fe	N	O	0	0
			43	34	1	4	4		

- Molecule 5 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
5	A	286	Total 286	O 286	0	0
5	B	27	Total 27	O 27	0	0
5	C	234	Total 234	O 234	0	0

3 Residue-property plots ⓘ

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. 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. 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: cytochrome c peroxidase
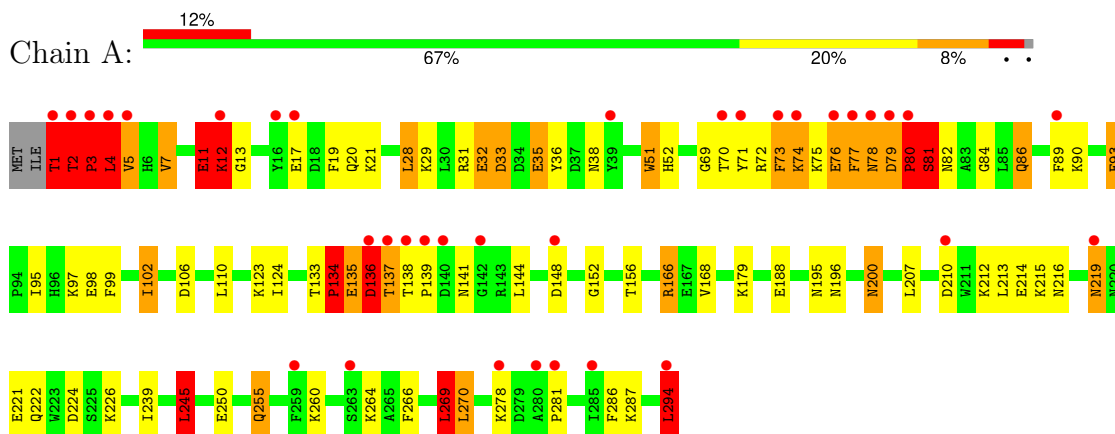
- Molecule 1: cytochrome c peroxidase
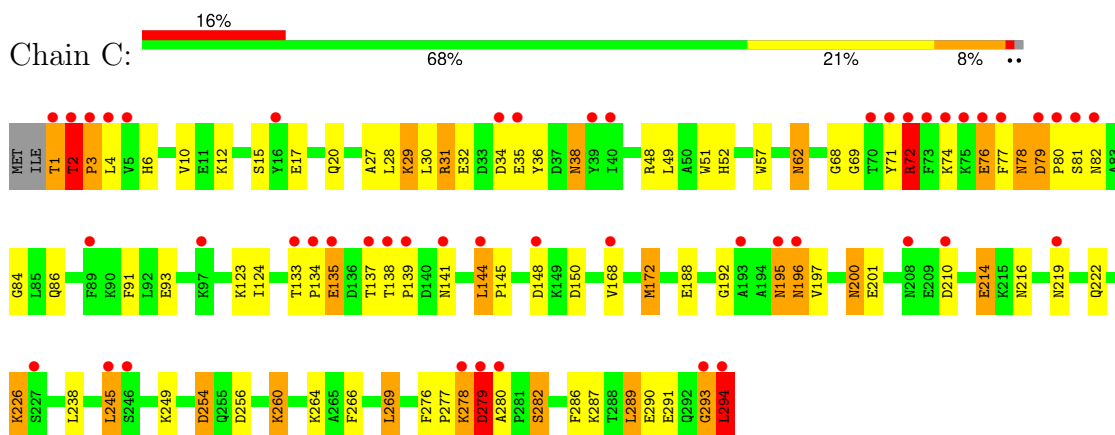
- Molecule 2: Cytochrome c iso-1
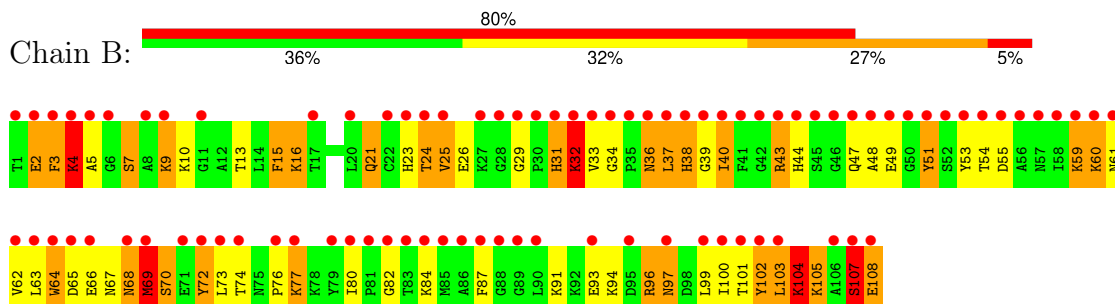
4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1	Depositor
Cell constants a, b, c, α, β, γ	44.53Å 47.09Å 84.29Å 102.47° 98.74° 102.16°	Depositor
Resolution (Å)	19.61 – 1.70 19.61 – 1.70	Depositor EDS
% Data completeness (in resolution range)	100.0 (19.61-1.70) 93.1 (19.61-1.70)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$<I/\sigma(I)>$ [1]	3.00 (at 1.70Å)	Xtriage
Refinement program	REFMAC	Depositor
R, R_{free}	0.219 , 0.230 0.225 , 0.237	Depositor DCC
R_{free} test set	3335 reflections (5.09%)	wwPDB-VP
Wilson B-factor (Å^2)	19.1	Xtriage
Anisotropy	0.068	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.37 , 48.9	EDS
L-test for twinning[2]	$<|L|> = 0.48$, $<L^2> = 0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.92	EDS
Total number of atoms	6265	wwPDB-VP
Average B, all atoms (Å^2)	27.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 6.46% of the height of the origin peak. No significant pseudotranslation is detected.*

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[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: HEC, ZNH

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	1.26	11/2438 (0.5%)	1.15	43/3302 (1.3%)
1	C	0.98	4/2438 (0.2%)	1.38	40/3302 (1.2%)
2	B	0.82	5/865 (0.6%)	1.47	35/1156 (3.0%)
All	All	1.09	20/5741 (0.3%)	1.30	118/7760 (1.5%)

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
1	A	0	8
1	C	1	6
All	All	1	14

The worst 5 of 20 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	1	THR	C-N	-30.02	0.65	1.34
1	A	79	ASP	C-N	21.35	1.74	1.34
1	A	2	THR	C-N	18.65	1.69	1.34
1	A	11	GLU	C-N	17.40	1.74	1.34
1	C	79	ASP	C-N	16.80	1.66	1.34

The worst 5 of 118 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	C	279	ASP	O-C-N	-39.65	59.26	122.70
1	C	294	LEU	CB-CA-C	-19.94	72.31	110.20
1	A	1	THR	O-C-N	-17.22	95.15	122.70

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Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	C	279	ASP	CB-CA-C	12.24	134.88	110.40
1	A	4	LEU	O-C-N	-11.80	103.82	122.70

All (1) chirality outliers are listed below:

Mol	Chain	Res	Type	Atom
1	C	279	ASP	CA

5 of 14 planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	1	THR	Mainchain,Peptide
1	A	136	ASP	Mainchain
1	A	3	PRO	Mainchain,Peptide
1	A	80	PRO	Mainchain
1	A	81	SER	Mainchain

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	2371	0	2246	118	4
1	C	2371	0	2251	72	4
2	B	847	0	848	90	3
3	A	43	0	30	0	0
3	C	43	0	30	0	0
4	B	43	0	30	4	0
5	A	286	0	0	66	2
5	B	27	0	0	12	1
5	C	234	0	0	37	2
All	All	6265	0	5435	277	9

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 25.

The worst 5 of 277 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:2:THR:C	1:A:3:PRO:N	1.69	1.45
1:A:11:GLU:C	1:A:12:LYS:N	1.74	1.39
1:A:79:ASP:C	1:A:80:PRO:N	1.74	1.36
2:B:100:ILE:O	2:B:104:LYS:HG3	1.22	1.34
1:C:76:GLU:HB3	5:C:1082:HOH:O	1.17	1.34

The worst 5 of 9 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:226:LYS:NZ	5:A:1233:HOH:O[1_445]	0.39	1.81
5:C:1321:HOH:O	5:C:1332:HOH:O[1_655]	0.68	1.52
1:A:226:LYS:CE	5:A:1233:HOH:O[1_445]	1.14	1.06
1:A:1:THR:OG1	5:C:1472:HOH:O[1_564]	1.86	0.34
1:A:17:GLU:OE1	2:B:49:GLU:OE1[1_665]	1.87	0.33

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	292/296 (99%)	281 (96%)	5 (2%)	6 (2%)	5	1
1	C	292/296 (99%)	284 (97%)	6 (2%)	2 (1%)	19	7
2	B	106/108 (98%)	97 (92%)	6 (6%)	3 (3%)	4	0
All	All	690/700 (99%)	662 (96%)	17 (2%)	11 (2%)	8	1

5 of 11 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	4	LEU
1	C	279	ASP
1	C	280	ALA
1	A	2	THR

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Mol	Chain	Res	Type
2	B	66	GLU

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	252/254 (99%)	233 (92%)	19 (8%)	11	2
1	C	252/254 (99%)	229 (91%)	23 (9%)	7	1
2	B	89/89 (100%)	72 (81%)	17 (19%)	1	0
All	All	593/597 (99%)	534 (90%)	59 (10%)	6	1

5 of 59 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	B	68	ASN
1	C	269	LEU
2	B	107	SER
1	C	260	LYS
1	C	196	ASN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 27 such sidechains are listed below:

Mol	Chain	Res	Type
2	B	61	ASN
1	C	20	GLN
1	C	208	ASN
1	C	6	HIS
1	C	24	ASN

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 oligosaccharides in this entry.

5.6　Ligand geometry　ⓘ

3 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	Bond lengths			Bond angles		
					Counts	RMSZ	$\#|Z| > 2$	Counts	RMSZ	$\#|Z| > 2$
3	ZNH	C	295	1	46,50,50	1.67	11 (23%)	55,82,82	1.43	6 (10%)
4	HEC	B	109	2	32,50,50	1.45	5 (15%)	30,82,82	1.33	4 (13%)
3	ZNH	A	295	1	46,50,50	1.21	5 (10%)	55,82,82	1.86	10 (18%)

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
3	ZNH	C	295	1	1/1/3/9	2/12/54/54	-
4	HEC	B	109	2	-	2/10/54/54	-
3	ZNH	A	295	1	1/1/3/9	4/12/54/54	-

The worst 5 of 21 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	C	295	ZNH	C1A-C2A	-3.70	1.38	1.45
3	C	295	ZNH	ZN-NB	3.48	2.12	1.99
4	B	109	HEC	CBB-CAB	-3.19	1.37	1.49

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Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	C	295	ZNH	CMC-C2C	3.15	1.57	1.51
3	C	295	ZNH	CHC-C4B	3.05	1.42	1.34

The worst 5 of 20 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	A	295	ZNH	CMC-C2C-C1C	-5.25	120.76	128.46
3	A	295	ZNH	C4B-C3B-C2B	-4.86	102.82	107.28
3	A	295	ZNH	CAD-C3D-C4D	4.85	133.16	124.70
3	A	295	ZNH	CAD-C3D-C2D	-4.34	119.74	127.87
3	C	295	ZNH	C4B-C3B-C2B	-4.33	103.30	107.28

All (2) chirality outliers are listed below:

Mol	Chain	Res	Type	Atom
3	A	295	ZNH	NA
3	C	295	ZNH	NA

5 of 8 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	A	295	ZNH	C4D-C3D-CAD-CBD
3	A	295	ZNH	C2D-C3D-CAD-CBD
3	C	295	ZNH	CAA-CBA-CGA-O2A
3	A	295	ZNH	CAA-CBA-CGA-O2A
3	A	295	ZNH	CAA-CBA-CGA-O1A

There are no ring outliers.

1 monomer is involved in 4 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	B	109	HEC	4	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 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
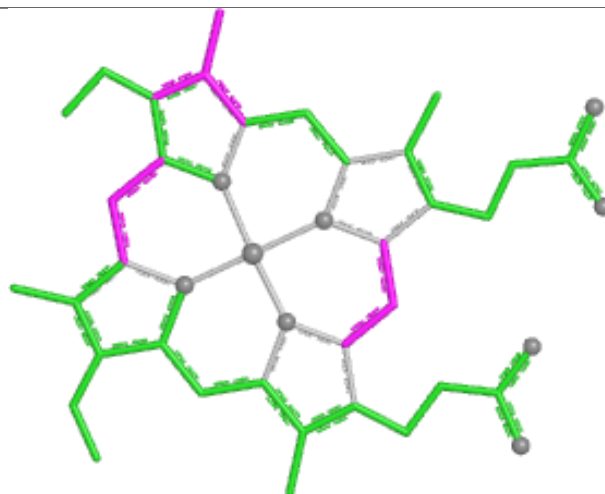
any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.
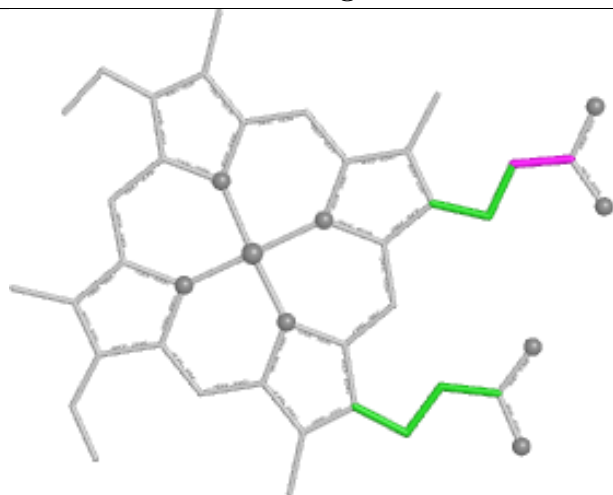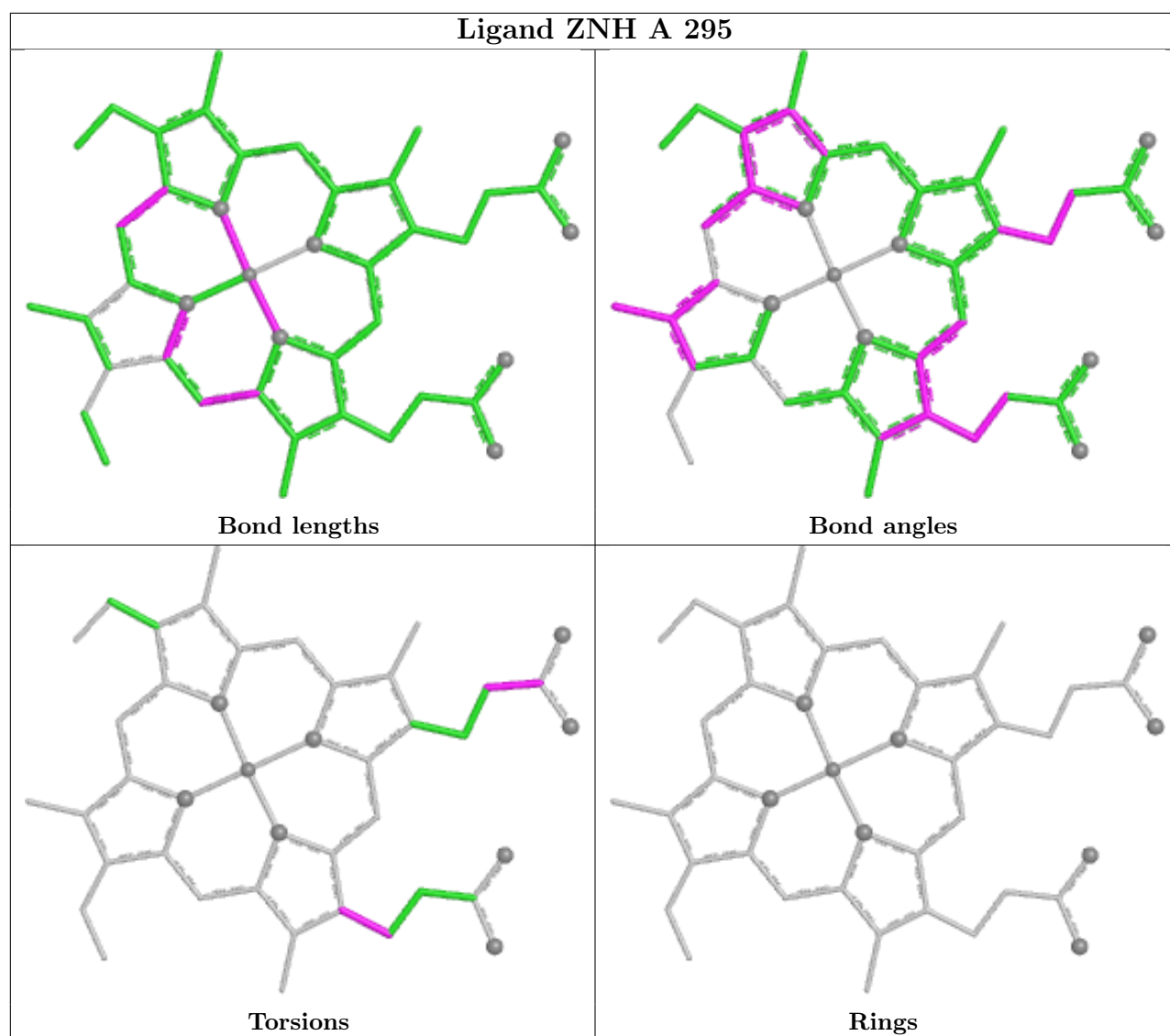
Ligand ZNH C 295

Bond lengths

Bond angles
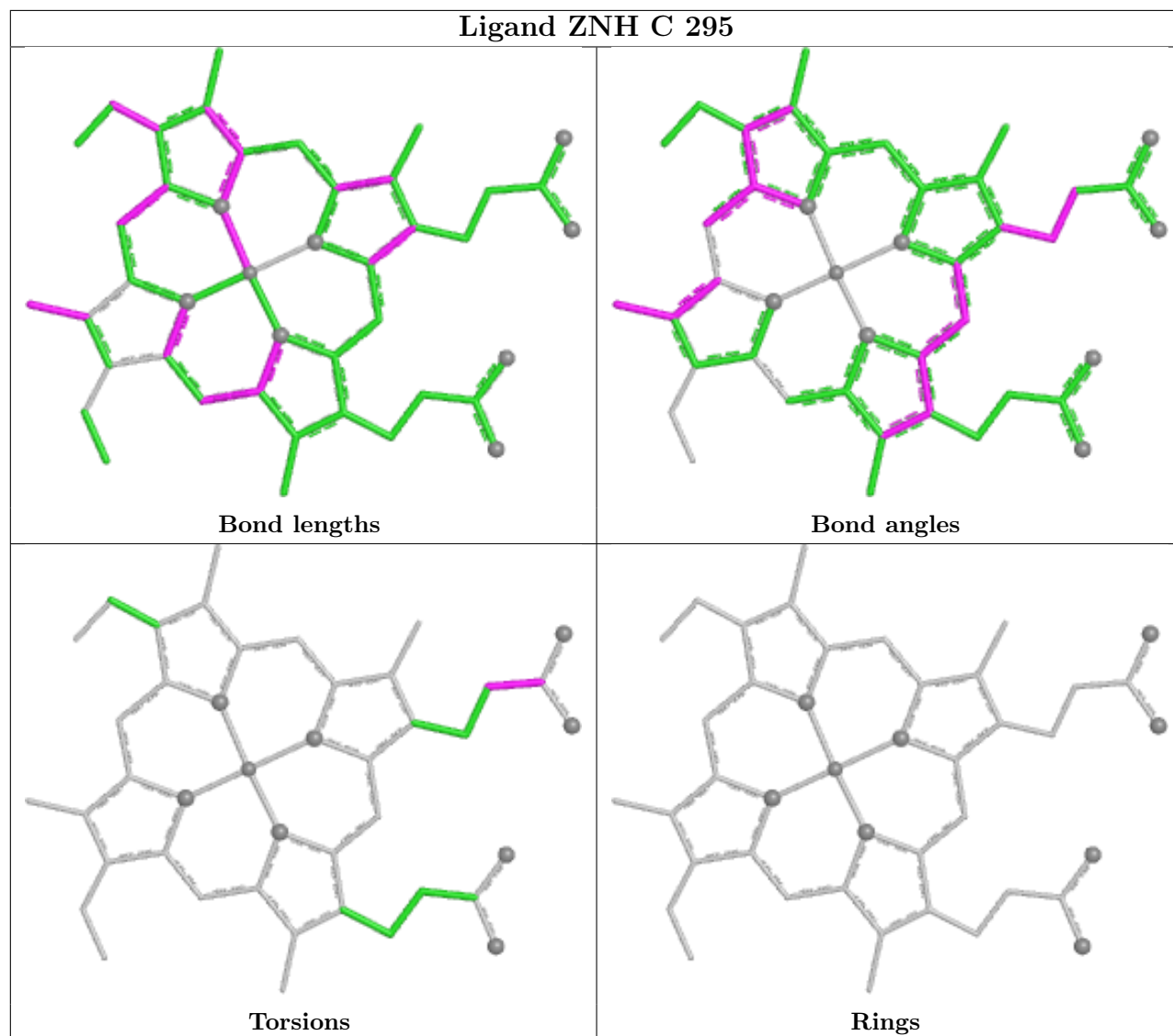
Torsions

Rings

Ligand HEC B 109

Bond lengths

Bond angles

Torsions

Rings

Ligand ZNH A 295
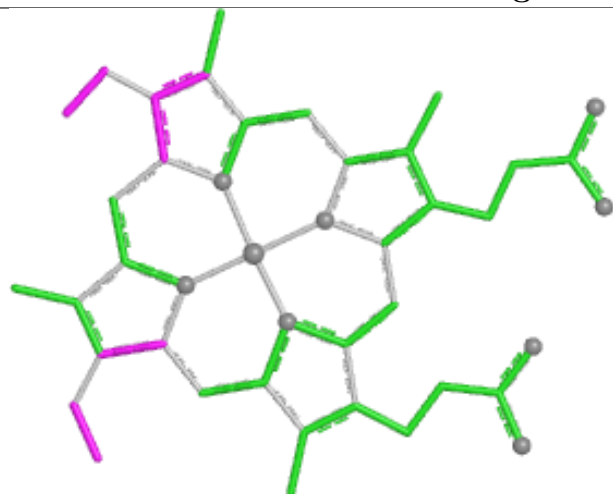
Bond lengths

Bond angles

Torsions

Rings

5.7 Other polymers ⓘ

There are no such residues in this entry.

5.8 Polymer linkage issues ⓘ

The following chains have linkage breaks:

Mol	Chain	Number of breaks
1	A	7
1	C	2
2	B	1

The worst 5 of 10 chain breaks are listed below:

Model	Chain	Residue-1	Atom-1	Residue-2	Atom-2	Distance (Å)
1	A	11:GLU	C	12:LYS	N	1.74
1	A	79:ASP	C	80:PRO	N	1.74
1	A	2:THR	C	3:PRO	N	1.69
1	C	79:ASP	C	80:PRO	N	1.66
1	C	277:PRO	C	278:LYS	N	1.65

6 Fit of model and data ⓘ

6.1 Protein, DNA and RNA chains ⓘ

Warning: The R factor obtained from EDS is 0.2691, which does not match the depositor's R factor of 0.219. Please interpret the results in this section carefully.

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(Å²)	Q<0.9
1	A	294/296 (99%)	0.96	35 (11%)	10	9	20, 24, 36, 50	0
1	C	294/296 (99%)	1.08	48 (16%)	5	5	18, 24, 39, 49	0
2	B	108/108 (100%)	2.87	86 (79%)	0	0	18, 34, 49, 50	0
All	All	696/700 (99%)	1.31	169 (24%)	2	2	18, 25, 43, 50	0

The worst 5 of 169 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	2	THR	7.4
2	B	55	ASP	6.7
2	B	3	PHE	6.5
1	C	138	THR	6.1
2	B	58	ILE	5.9

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

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

6.3 Carbohydrates ⓘ

There are no monosaccharides in this entry.

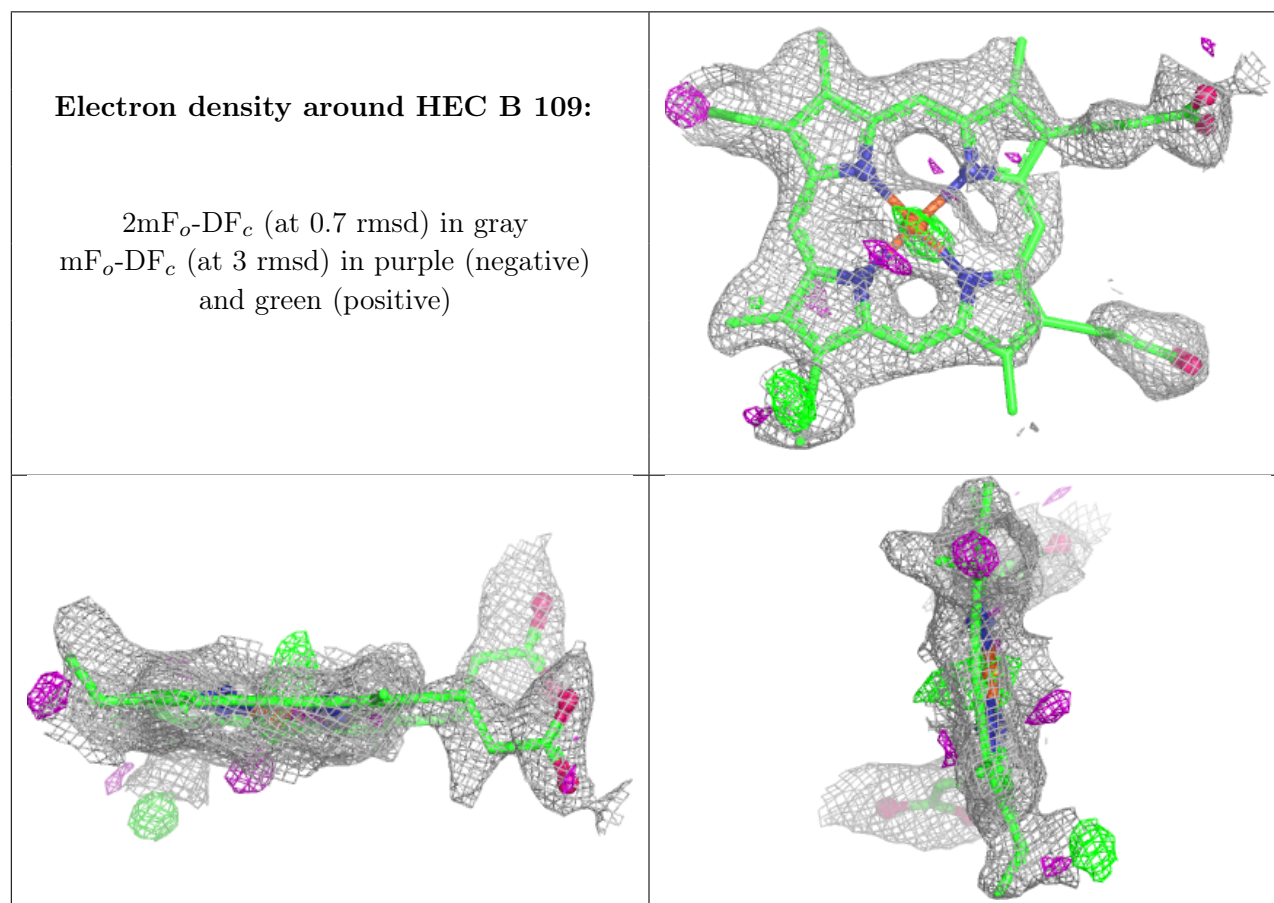## 6.4 Ligands ⓘ

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.
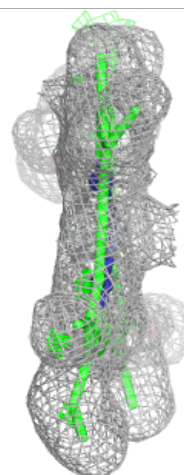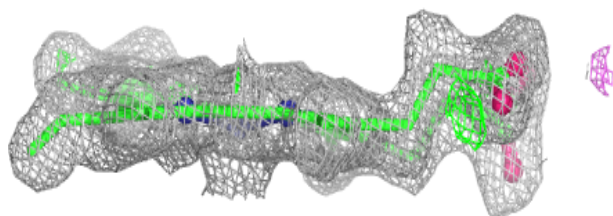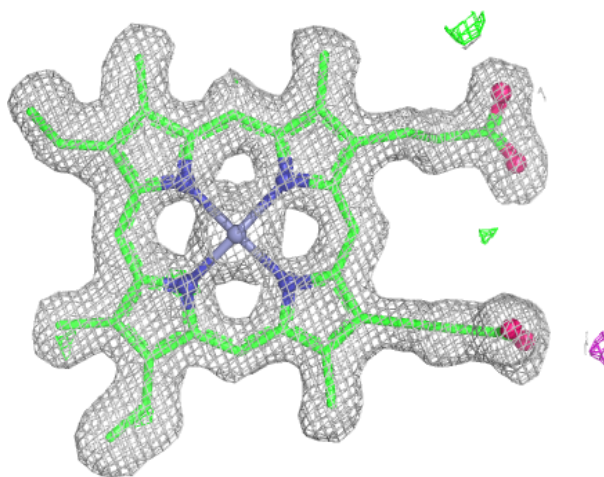
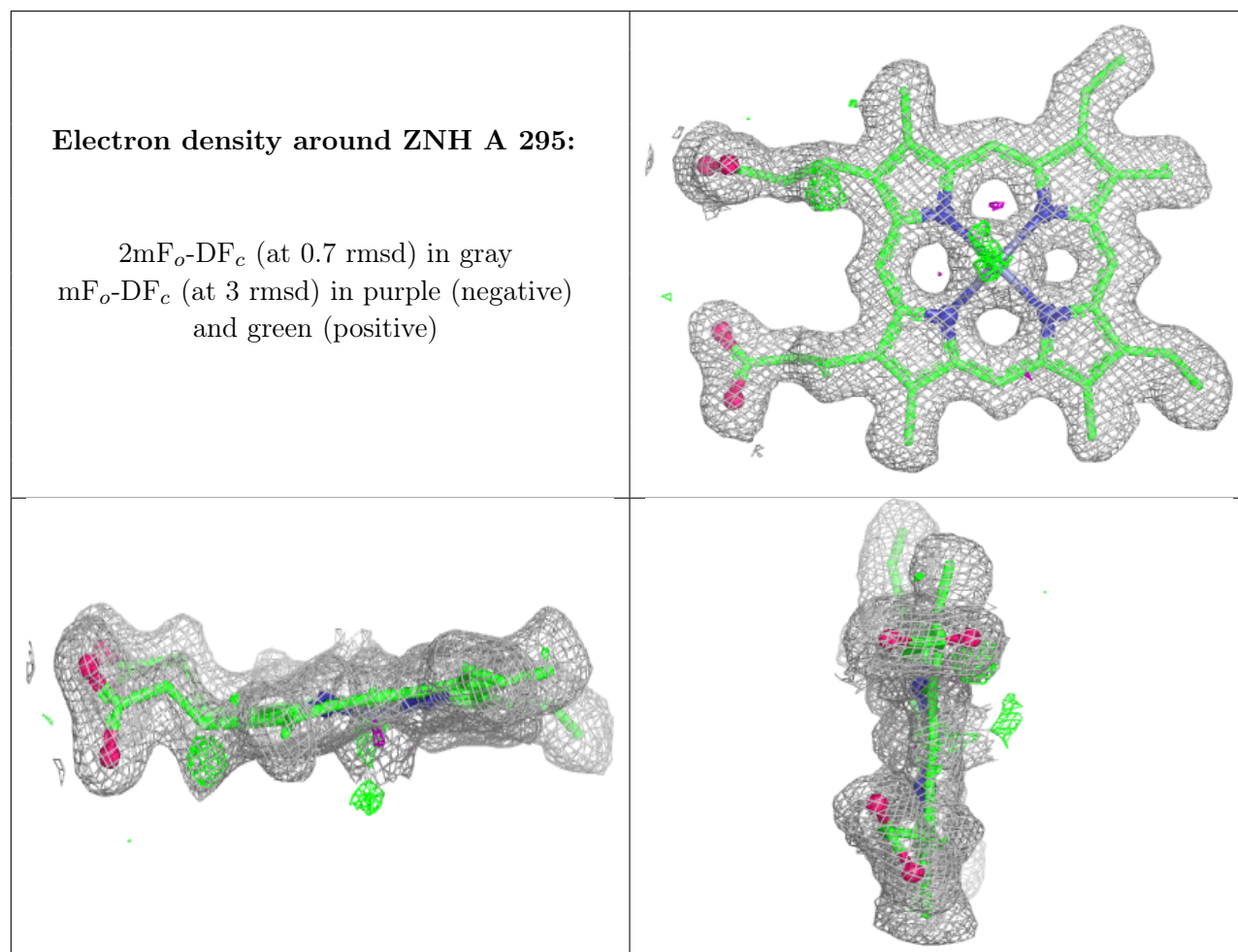| Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å^2)	Q<0.9
4	HEC	B	109	43/43	0.83	0.13	17,20,21,23	0
3	ZNH	C	295	43/43	0.97	0.09	19,21,23,26	0
3	ZNH	A	295	43/43	0.98	0.08	19,22,26,28	0

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.

Electron density around HEC B 109:

$2mF_o$-DF_c (at 0.7 rmsd) in gray
mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)

Electron density around ZNH C 295:

$2mF_o$-DF_c (at 0.7 rmsd) in gray
mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)

Electron density around ZNH A 295:

$2mF_o$-DF_c (at 0.7 rmsd) in gray
mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)

6.5 Other polymers ⓘ

There are no such residues in this entry.