Full wwPDB X-ray Structure Validation Report (i)

Oct 2, 2023 – 10:14 AM EDT

PDB ID	:	6NG1
Title	:	Structure of human neuronal nitric oxide synthase R354A/G357D mutant heme domain in complex with 6-(3-fluoro-5-(3-(methylamino)prop-1-yn-1-yl)phenethyl)-4-methylpyridin-2-amine
Authors	:	Li, H.; Poulos, T.L.
Deposited on	:	2018-12-21
Resolution	:	2.15 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	:	FAILED
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	FAILED
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.35.1

1 Overall quality at a glance (i)

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

The reported resolution of this entry is 2.15 Å.

There are no overall percentile quality scores available for this entry.

MolProbity and EDS failed to run properly - the sequence quality summary graphics cannot be shown.

2 Entry composition (i)

There are 6 unique types of molecules in this entry. The entry contains 7126 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.

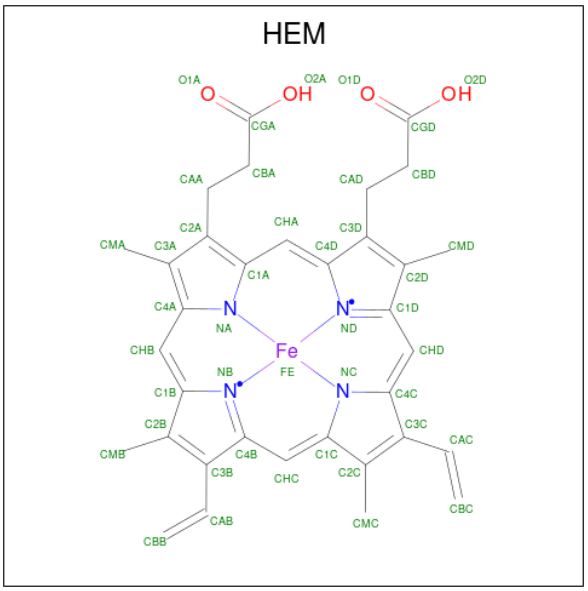
- Molecule 1 is a protein called Nitric oxide synthase, brain.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	413	3371	2159	574	616	22	0	2	0
1	B	410	3352	2147	571	612	22	0	3	0

There are 4 discrepancies between the modelled and reference sequences:

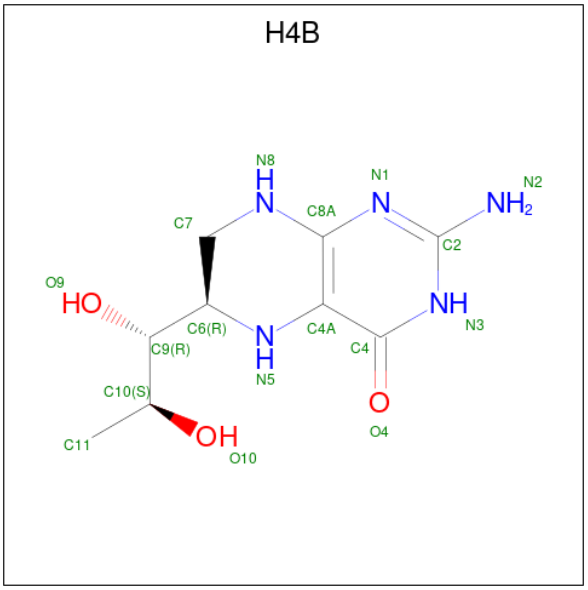
Chain	Residue	Modelled	Actual	Comment	Reference
A	354	ALA	ARG	engineered mutation	UNP P29475
A	357	ASP	GLY	engineered mutation	UNP P29475
B	354	ALA	ARG	engineered mutation	UNP P29475
B	357	ASP	GLY	engineered mutation	UNP P29475

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



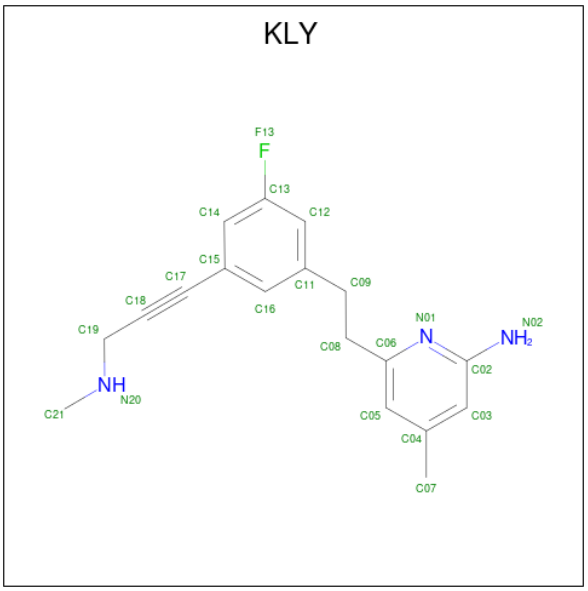
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
			Total	C	Fe	N	O		
2	A	1	43	34	1	4	4	0	0
2	B	1	43	34	1	4	4	0	0

- 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
3	B	1	17	9	5	3		0	0

- Molecule 4 is 6-(2-{3-fluoro-5-[3-(methylamino)prop-1-yn-1-yl]phenyl}ethyl)-4-methylpyridin-2-amine (three-letter code: KLY) (formula: $C_{18}H_{20}FN_3$).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
			Total	C	F	N		
4	A	1	22	18	1	3	0	0
			Total	C	F	N		
4	B	1	22	18	1	3	0	0

- Molecule 5 is ZINC ION (three-letter code: ZN) (formula: Zn).

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

- Molecule 6 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
6	A	144	144	144	0	0
			Total	O		
6	B	94	94	94	0	0

MolProbity and EDS failed to run properly - this section is therefore empty.

3 Data and refinement statistics (i)

EDS failed to run properly - this section is therefore incomplete.

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, α, β, γ	52.24Å 123.35Å 163.98Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	49.97 – 2.15	Depositor
% Data completeness (in resolution range)	97.4 (49.97-2.15)	Depositor
R_{merge}	0.16	Depositor
R_{sym}	0.16	Depositor
$< I/\sigma(I) >$ [1]	1.27 (at 2.14Å)	Xtriage
Refinement program	PHENIX 1.9_1692	Depositor
R, R_{free}	0.204 , 0.256	Depositor
Wilson B-factor (Å^2)	41.5	Xtriage
Anisotropy	0.627	Xtriage
L-test for twinning[2]	$< \|L\| > = 0.49$, $< L^2 > = 0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
Total number of atoms	7126	wwPDB-VP
Average B, all atoms (Å^2)	64.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.23% 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.

4 Model quality (i)

4.1 Standard geometry (i)

MolProbity failed to run properly - this section is therefore empty.

4.2 Too-close contacts (i)

MolProbity failed to run properly - this section is therefore empty.

4.3 Torsion angles (i)

4.3.1 Protein backbone (i)

MolProbity failed to run properly - this section is therefore empty.

4.3.2 Protein sidechains (i)

MolProbity failed to run properly - this section is therefore empty.

4.3.3 RNA (i)

MolProbity failed to run properly - this section is therefore empty.

4.4 Non-standard residues in protein, DNA, RNA chains (i)

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

4.5 Carbohydrates (i)

There are no monosaccharides in this entry.

4.6 Ligand geometry (i)

Of 7 ligands modelled in this entry, 1 is monoatomic - leaving 6 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	801	1	41,50,50	1.81	5 (12%)	45,82,82	1.75	8 (17%)
4	KLY	B	803	-	22,23,23	1.40	1 (4%)	28,30,30	1.81	6 (21%)
4	KLY	A	803	-	22,23,23	1.30	1 (4%)	28,30,30	1.69	4 (14%)
3	H4B	B	802	-	16,18,18	0.84	0	11,26,26	2.61	5 (45%)
2	HEM	B	801	1	41,50,50	1.88	5 (12%)	45,82,82	1.85	11 (24%)
3	H4B	A	802	-	16,18,18	0.85	0	11,26,26	2.57	5 (45%)

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	801	1	-	0/12/54/54	-
4	KLY	B	803	-	-	1/8/10/10	0/2/2/2
4	KLY	A	803	-	-	3/8/10/10	0/2/2/2
3	H4B	B	802	-	-	0/8/17/17	0/2/2/2
2	HEM	B	801	1	-	1/12/54/54	-
3	H4B	A	802	-	-	0/8/17/17	0/2/2/2

All (12) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	B	801	HEM	C3D-C2D	7.45	1.52	1.36
2	A	801	HEM	C3D-C2D	7.07	1.51	1.36
4	B	803	KLY	C15-C17	-6.18	1.29	1.44
4	A	803	KLY	C15-C17	-5.59	1.30	1.44
2	A	801	HEM	C3C-C2C	-4.07	1.34	1.40
2	B	801	HEM	C3C-CAC	3.83	1.55	1.47
2	B	801	HEM	C3C-C2C	-3.70	1.35	1.40
2	A	801	HEM	C3C-CAC	3.40	1.54	1.47
2	B	801	HEM	CAB-C3B	2.87	1.55	1.47
2	A	801	HEM	CAB-C3B	2.85	1.55	1.47
2	B	801	HEM	CMD-C2D	2.46	1.56	1.50
2	A	801	HEM	C3B-C2B	-2.23	1.32	1.37

All (39) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	A	801	HEM	CBA-CAA-C2A	-6.26	101.94	112.62
3	B	802	H4B	C8A-C4A-C4	5.60	119.55	114.57
2	B	801	HEM	C4D-ND-C1D	5.50	110.75	105.07
4	B	803	KLY	C09-C08-C06	-5.42	100.85	112.99
2	B	801	HEM	C4B-CHC-C1C	5.15	129.35	122.56
4	A	803	KLY	C09-C08-C06	-5.11	101.54	112.99
3	A	802	H4B	C8A-C4A-C4	5.07	119.08	114.57
4	A	803	KLY	C02-N01-C06	4.42	121.45	118.10
2	B	801	HEM	CBA-CAA-C2A	-4.19	105.47	112.62
2	A	801	HEM	C4B-CHC-C1C	4.03	127.87	122.56
4	B	803	KLY	C02-N01-C06	3.76	120.95	118.10
2	A	801	HEM	C4D-ND-C1D	3.67	108.87	105.07
3	A	802	H4B	C2-N3-C4	3.16	120.95	115.93
3	B	802	H4B	C4-C4A-N5	3.15	121.77	119.12
3	A	802	H4B	N1-C2-N3	-3.13	120.51	125.42
3	B	802	H4B	C2-N3-C4	3.12	120.89	115.93
3	A	802	H4B	C4-C4A-N5	3.03	121.66	119.12
3	B	802	H4B	N1-C2-N3	-2.93	120.83	125.42
4	B	803	KLY	C08-C06-N01	2.90	120.28	115.95
4	B	803	KLY	C12-C13-C14	-2.90	119.85	123.52
2	B	801	HEM	CMD-C2D-C1D	2.86	129.39	125.04
2	B	801	HEM	CAD-C3D-C4D	2.85	129.64	124.66
2	A	801	HEM	CBD-CAD-C3D	-2.85	104.71	112.63
4	B	803	KLY	C05-C06-N01	-2.78	119.96	122.90
2	B	801	HEM	CAD-CBD-CGD	-2.67	107.85	113.60
3	A	802	H4B	C2-N1-C8A	2.61	120.39	114.54
2	A	801	HEM	C3B-C2B-C1B	2.57	108.39	106.49
3	B	802	H4B	C2-N1-C8A	2.55	120.25	114.54
4	A	803	KLY	C12-C13-C14	-2.42	120.46	123.52
2	B	801	HEM	CMC-C2C-C3C	2.41	129.18	124.68
2	A	801	HEM	CAD-C3D-C4D	2.33	128.72	124.66
4	A	803	KLY	C05-C06-N01	-2.30	120.46	122.90
2	B	801	HEM	CBD-CAD-C3D	-2.27	106.32	112.63
2	B	801	HEM	C1D-C2D-C3D	-2.23	104.61	106.96
2	A	801	HEM	C1D-C2D-C3D	-2.18	104.66	106.96
2	A	801	HEM	CMC-C2C-C3C	2.10	128.60	124.68
4	B	803	KLY	N02-C02-N01	2.08	119.78	116.49
2	B	801	HEM	CAD-C3D-C2D	-2.05	124.06	127.88
2	B	801	HEM	C3B-C2B-C1B	2.01	107.98	106.49

There are no chirality outliers.

All (5) torsion outliers are listed below:

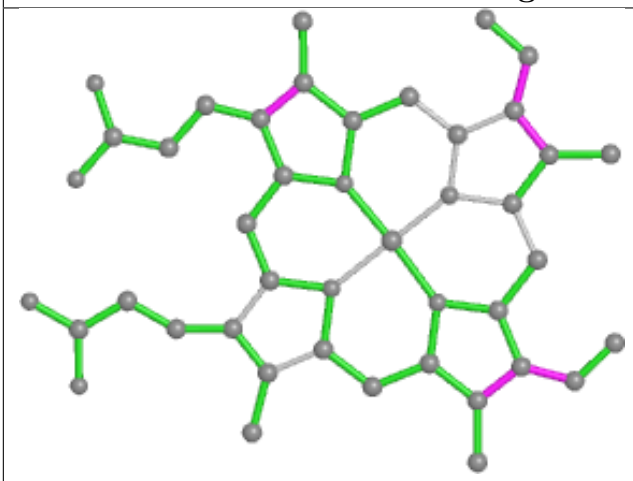
Mol	Chain	Res	Type	Atoms
4	A	803	KLY	C18-C19-N20-C21
4	B	803	KLY	C18-C19-N20-C21
2	B	801	HEM	C4B-C3B-CAB-CBB
4	A	803	KLY	C08-C09-C11-C16
4	A	803	KLY	C08-C09-C11-C12

There are no ring outliers.

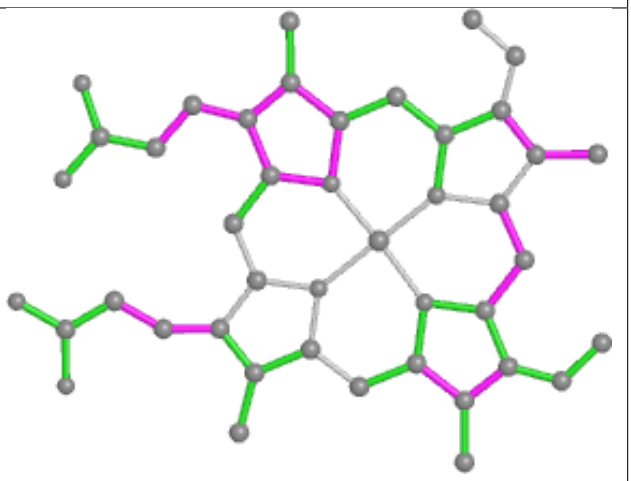
No monomer is involved in short contacts.

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.

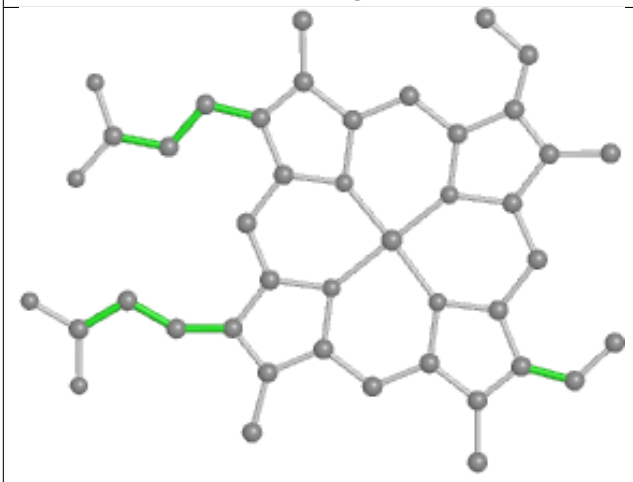
Ligand HEM A 801



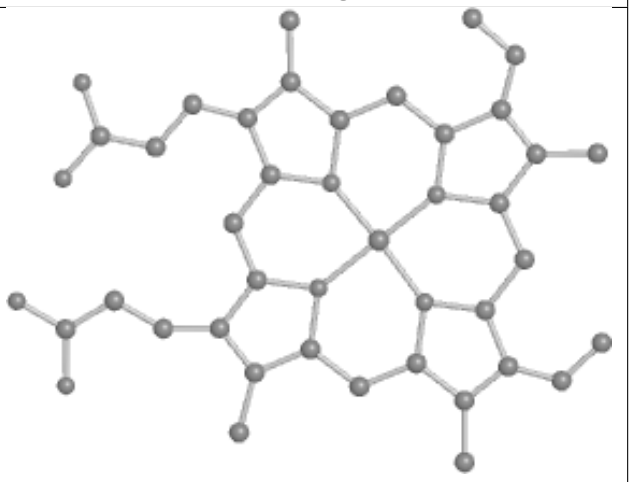
Bond lengths



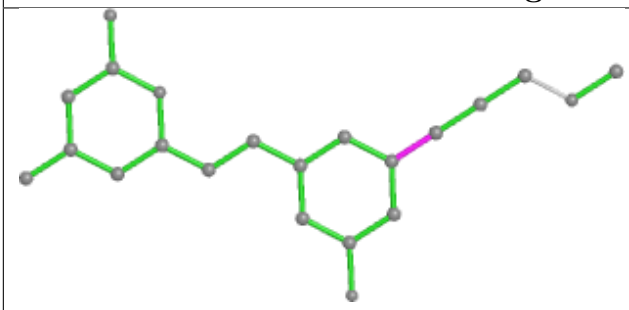
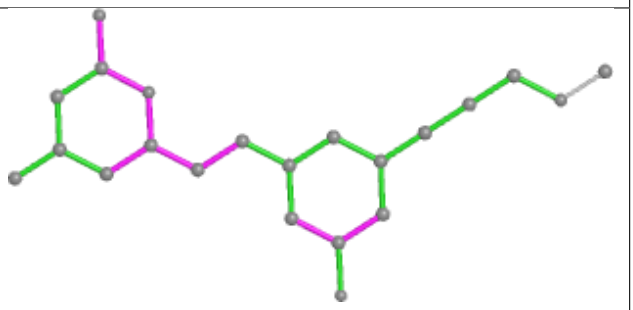
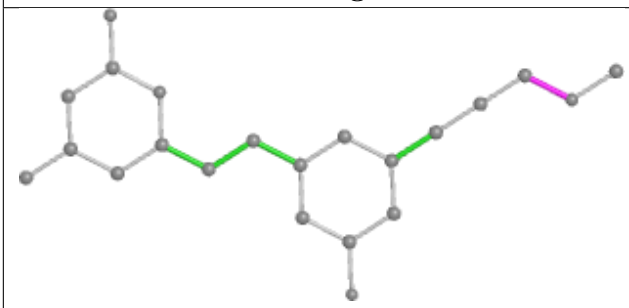
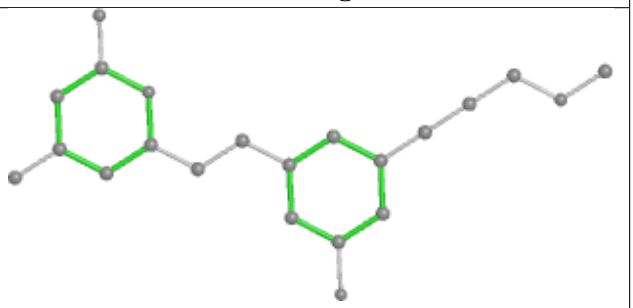
Bond angles

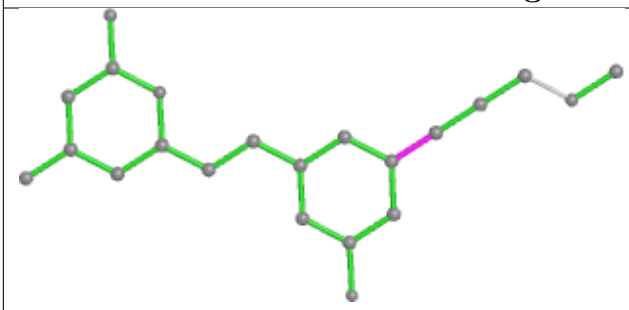
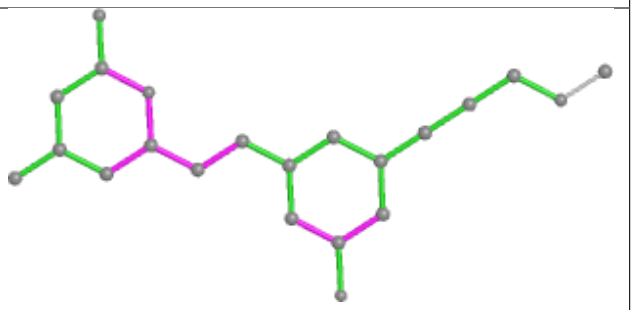
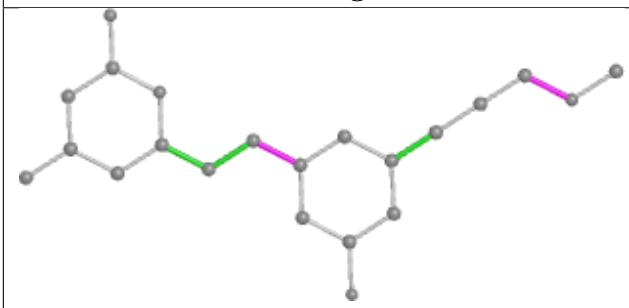
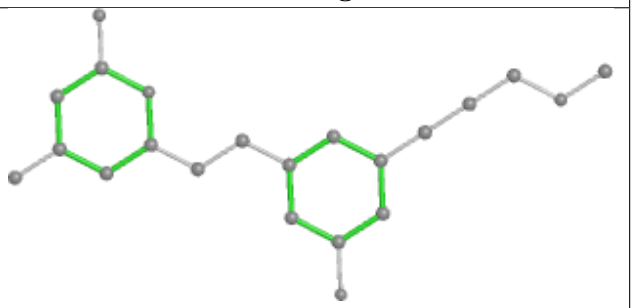


Torsions

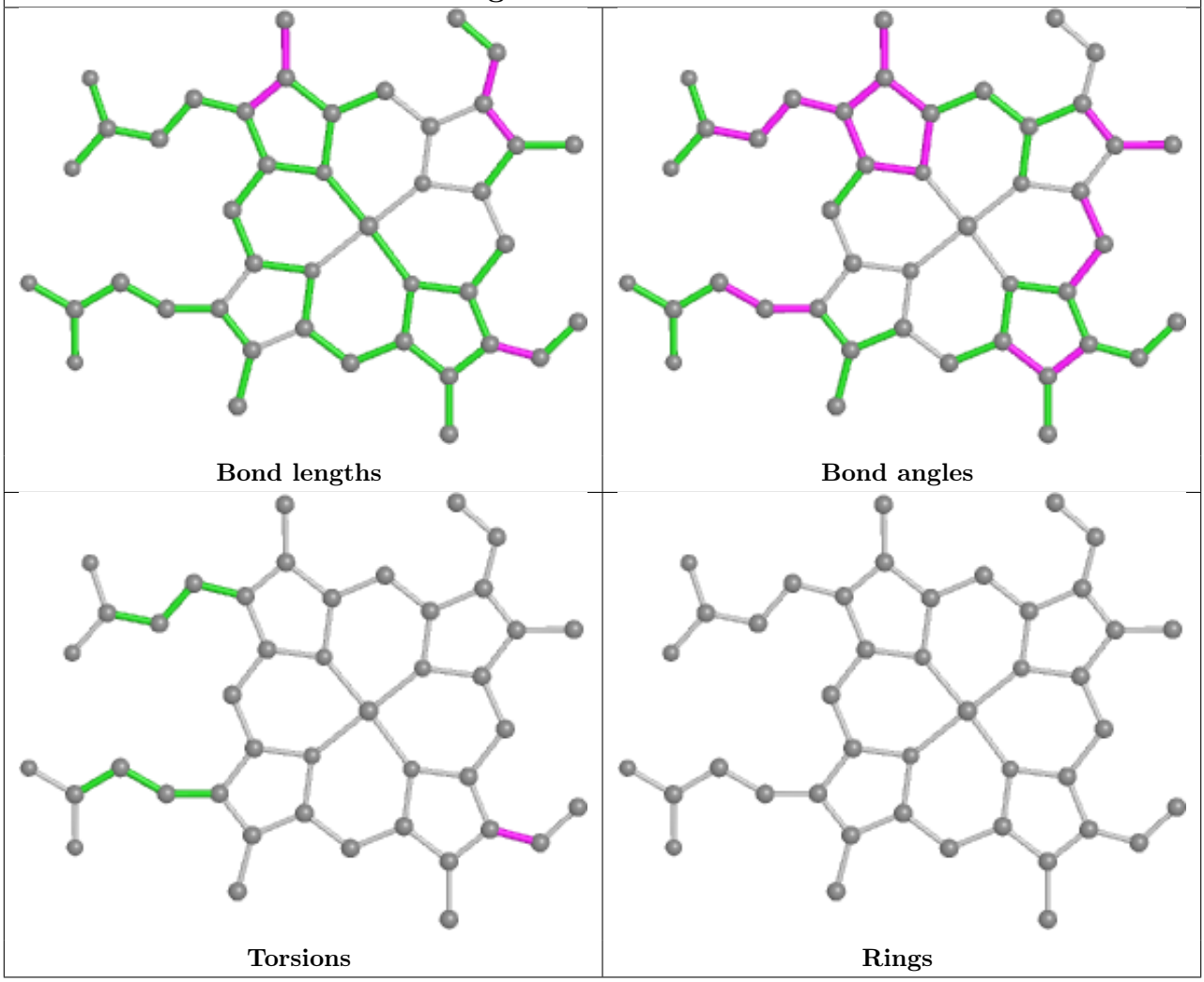


Rings

Ligand KLY B 803	
 Bond lengths	 Bond angles
 Torsions	 Rings

Ligand KLY A 803	
 Bond lengths	 Bond angles
 Torsions	 Rings

Ligand HEM B 801



4.7 Other polymers (i)

There are no such residues in this entry.

4.8 Polymer linkage issues (i)

There are no chain breaks in this entry.

5 Fit of model and data (i)

5.1 Protein, DNA and RNA chains (i)

EDS failed to run properly - this section is therefore empty.

5.2 Non-standard residues in protein, DNA, RNA chains (i)

EDS failed to run properly - this section is therefore empty.

5.3 Carbohydrates (i)

EDS failed to run properly - this section is therefore empty.

5.4 Ligands (i)

EDS failed to run properly - this section is therefore empty.

5.5 Other polymers (i)

EDS failed to run properly - this section is therefore empty.