W O R L D W I D E
wwPDB
PROTEIN DATA BANK

wwPDB X-ray Structure Validation Summary Report (i)

Sep 13, 2023 – 05:26 pm BST

PDB ID	:	8BYJ
Title	:	The structures of Ace2 in complex with bicyclic peptide inhibitor
Authors	:	Brear, P.; Lulla, A.; Harman, M.; Dods, R.; Chen, L.; Bezerra, G.; Demydchuk, Y.; Stanway, S.; Hyvonen, M.
Deposited on	:	2022-12-13
Resolution	:	2.07 Å(reported)

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

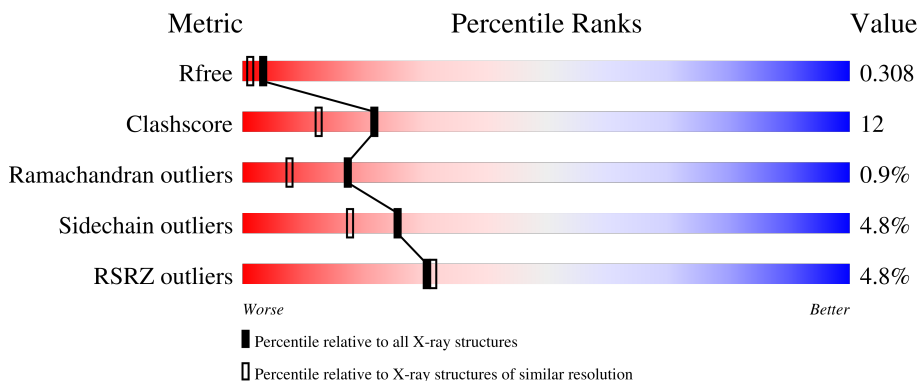|		
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.35.1
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.35.1

1 Overall quality at a glance ⓘ

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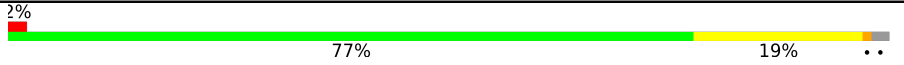*X-RAY DIFFRACTION*

The reported resolution of this entry is 2.07 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		0.308
Clashscore		12
Ramachandran outliers		0.9%
Sidechain outliers		4.8%
RSRZ outliers		4.8%

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}	130704	2684 (2.08-2.04)
Clashscore	141614	2801 (2.08-2.04)
Ramachandran outliers	138981	2768 (2.08-2.04)
Sidechain outliers	138945	2768 (2.08-2.04)
RSRZ outliers	127900	2646 (2.08-2.04)

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% 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	609	2% / 77% / 19% / · ·
1	B	609	7% / 62% / 33% / · ·
2	C	18	11% / 56% / 33% / 11%
2	D	18	6% / 56% / 33% / 6% / 6%

2 Entry composition ⓘ

There are 6 unique types of molecules in this entry. The entry contains 10139 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 Processed angiotensin-converting enzyme 2.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	596	Total 4862	C 3111	N 805	O 917	S 29	0	0	0
1	B	595	Total 4856	C 3108	N 804	O 915	S 29	0	0	0

There are 24 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	18	GLY	-	expression tag	UNP Q9BYF1
A	616	SER	-	expression tag	UNP Q9BYF1
A	617	SER	-	expression tag	UNP Q9BYF1
A	618	PRO	-	expression tag	UNP Q9BYF1
A	619	HIS	-	expression tag	UNP Q9BYF1
A	620	HIS	-	expression tag	UNP Q9BYF1
A	621	HIS	-	expression tag	UNP Q9BYF1
A	622	HIS	-	expression tag	UNP Q9BYF1
A	623	HIS	-	expression tag	UNP Q9BYF1
A	624	HIS	-	expression tag	UNP Q9BYF1
A	625	HIS	-	expression tag	UNP Q9BYF1
A	626	HIS	-	expression tag	UNP Q9BYF1
B	18	GLY	-	expression tag	UNP Q9BYF1
B	616	SER	-	expression tag	UNP Q9BYF1
B	617	SER	-	expression tag	UNP Q9BYF1
B	618	PRO	-	expression tag	UNP Q9BYF1
B	619	HIS	-	expression tag	UNP Q9BYF1
B	620	HIS	-	expression tag	UNP Q9BYF1
B	621	HIS	-	expression tag	UNP Q9BYF1
B	622	HIS	-	expression tag	UNP Q9BYF1
B	623	HIS	-	expression tag	UNP Q9BYF1
B	624	HIS	-	expression tag	UNP Q9BYF1
B	625	HIS	-	expression tag	UNP Q9BYF1
B	626	HIS	-	expression tag	UNP Q9BYF1

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- Molecule 2 is a protein called ALA-CYS-VAL-ARG-SER-HIS-CYS-SER-SER-LEU-LEU-PRO-ARG-ILE-HIS-CYS-ALA.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	C	18	Total 127	C 76	N 28	O 20	S 3	0	0	1
2	D	18	Total 127	C 76	N 28	O 20	S 3	0	0	1

- Molecule 3 is ZINC ION (three-letter code: ZN) (formula: Zn).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
3	A	1	Total 1	Zn 1	0	0
3	B	1	Total 1	Zn 1	0	0

- Molecule 4 is SODIUM ION (three-letter code: NA) (formula: Na).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
4	B	1	Total 1	Na 1	0	0

- Molecule 5 is 1-[3,5-bis(3-bromanylpropanoyl)-1,3,5-triazinan-1-yl]-3-bromanyl-propan-1-one (three-letter code: LFI) (formula: $C_{12}H_{18}Br_3N_3O_3$) (labeled as "Ligand of Interest" by depositor).
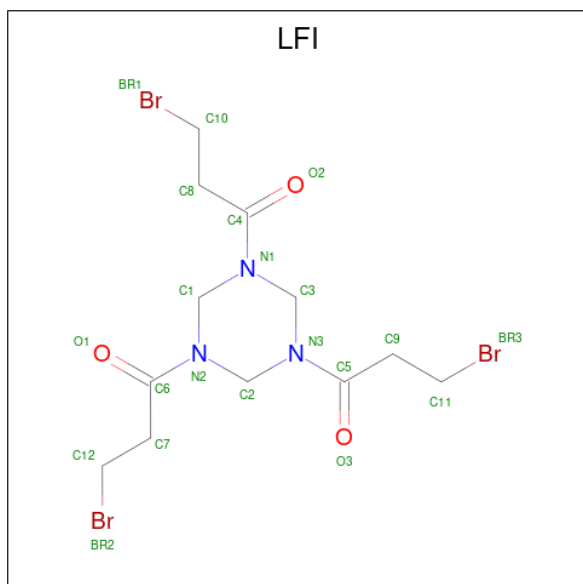
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
5	C	1	Total 18	C 12	N 3	O 3	0	0
5	D	1	Total 18	C 12	N 3	O 3	0	0

• Molecule 6 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
6	A	76	Total 76	O 76	0	0
6	B	47	Total 47	O 47	0	0
6	C	1	Total 1	O 1	0	0
6	D	4	Total 4	O 4	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 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: Processed angiotensin-converting enzyme 2

Chain A:

• Molecule 1: Processed angiotensin-converting enzyme 2

Chain B:

E457 K458 M462 P469 K470 D471 M474 K475 K476 W477 W478 K481 V491 C498 D499 P500 S511 F512 I513 R514 R518 Y521 Q522 F523 Q524 F525 Q526 L529 C530 A533 K534 H535 E536 G537 P538 L539 H540 K541 C542 D543 I544 T548 E549 A550 G551 Q552 K553 L554

F555 N556 M557 L560 E564 L568 A569 L570 E571 N572 V573 V574 G575 A576 K577 N578 M579 N580 V581 R582 P583 L584 L585 N586 Y587 F588 E589 P590 L591 F592 T593 W594 L595 Q598 N599 K600 N601 S602 F603 V604 A614 ASP SER SER PRO HIS HIS HIS HIS HIS HIS

• Molecule 2: ALA-CYS-VAL-ARG-SER-HIS-CYS-SER-SER-LEU-LEU-PRO-ARG-ILE-HIS-CYS-ALA

11%

Chain C: 56%   33%   11%

A0 C1 V2 R3 S4 H5 C6 R12 I13 A16 NH217

• Molecule 2: ALA-CYS-VAL-ARG-SER-HIS-CYS-SER-SER-LEU-LEU-PRO-ARG-ILE-HIS-CYS-ALA

6%

Chain D: 56%   33%   6%   6%

A0 C1 V2 R3 S4 H5 C6 S7 S8 R12 NH217

4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α, β, γ	78.78Å 75.96Å 114.91Å 90.00° 102.15° 90.00°	Depositor
Resolution (Å)	77.02 – 2.07 77.02 – 2.07	Depositor EDS
% Data completeness (in resolution range)	84.8 (77.02-2.07) 84.8 (77.02-2.07)	Depositor EDS
R_{merge}	0.08	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	0.98 (at 2.07Å)	Xtriage
Refinement program	REFMAC 5.8.0352	Depositor
R, R_{free}	0.224 , 0.304 0.233 , 0.308	Depositor DCC
R_{free} test set	3336 reflections (4.85%)	wwPDB-VP
Wilson B-factor (Å^2)	53.1	Xtriage
Anisotropy	0.561	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.32 , 47.9	EDS
L-test for twinning[2]	$< |L| > = 0.50, < L^2 > = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.96	EDS
Total number of atoms	10139	wwPDB-VP
Average B, all atoms (Å^2)	79.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 4.55% 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: LFI, ZN, NA, NH2

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	0.43	0/4999	0.68	0/6792
1	B	0.42	0/4993	0.68	0/6784
2	C	0.43	0/128	0.77	0/172
2	D	0.57	0/128	1.02	0/172
All	All	0.43	0/10248	0.69	0/13920

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	4
1	B	0	1
2	D	0	1
All	All	0	6

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

5 of 6 planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	204	ARG	Sidechain
1	A	219	ARG	Sidechain
1	A	393	ARG	Sidechain
1	A	559	ARG	Sidechain
1	B	518	ARG	Sidechain

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	4862	0	4639	74	0
1	B	4856	0	4633	153	0
2	C	127	0	128	6	0
2	D	127	0	128	5	0
3	A	1	0	0	0	0
3	B	1	0	0	0	0
4	B	1	0	0	0	0
5	C	18	0	0	1	0
5	D	18	0	0	2	0
6	A	76	0	0	3	0
6	B	47	0	0	3	0
6	C	1	0	0	0	0
6	D	4	0	0	0	0
All	All	10139	0	9528	233	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 12.

The worst 5 of 233 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:B:535:HIS:NE2	1:B:538:PRO:O	2.22	0.72
1:A:50:TYR:CE1	1:A:54:ILE:HG23	2.25	0.72
1:B:524:GLN:HB3	1:B:574:VAL:HG11	1.69	0.72
1:B:542:CYS:SG	1:B:544:ILE:HG23	2.29	0.72
1:A:168:TRP:CZ3	1:A:172:VAL:HG21	2.25	0.72

There are no symmetry-related clashes.

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	594/609 (98%)	569 (96%)	24 (4%)	1 (0%)	47	39
1	B	593/609 (97%)	548 (92%)	38 (6%)	7 (1%)	13	4
2	C	16/18 (89%)	13 (81%)	2 (12%)	1 (6%)	1	0
2	D	16/18 (89%)	9 (56%)	5 (31%)	2 (12%)	0	0
All	All	1219/1254 (97%)	1139 (93%)	69 (6%)	11 (1%)	17	8

5 of 11 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
2	C	2	VAL
2	D	2	VAL
2	D	7	SER
1	B	103	ASN
1	B	364	VAL

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	526/538 (98%)	510 (97%)	16 (3%)	41	35
1	B	525/538 (98%)	494 (94%)	31 (6%)	19	11
2	C	15/15 (100%)	12 (80%)	3 (20%)	1	0
2	D	15/15 (100%)	13 (87%)	2 (13%)	4	1
All	All	1081/1106 (98%)	1029 (95%)	52 (5%)	25	18

5 of 52 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	295	ASP
1	B	421	ILE

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Mol	Chain	Res	Type
2	C	12	ARG
1	B	314	PHE
1	B	359	LEU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 23 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	96	GLN
1	B	239	HIS
1	B	149	ASN
1	B	345	HIS
1	A	437	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 monosaccharides in this entry.

5.6 Ligand geometry ⓘ

Of 5 ligands modelled in this entry, 3 are monoatomic - leaving 2 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	LFI	C	101	2	18,18,21	0.65	0	24,24,27	1.24	3 (12%)

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	#|Z| > 2	Counts	RMSZ	#|Z| > 2
5	LFI	D	101	2	18,18,21	0.48	0	24,24,27	1.31	2 (8%)

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
5	LFI	C	101	2	-	11/18/30/33	0/0/1/1
5	LFI	D	101	2	-	13/18/30/33	0/0/1/1

There are no bond length outliers.

All (5) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
5	D	101	LFI	N3-C2-N2	-4.17	104.30	110.77
5	D	101	LFI	N3-C3-N1	-4.12	104.37	110.77
5	C	101	LFI	N3-C2-N2	-3.23	105.76	110.77
5	C	101	LFI	C3-N3-C2	2.60	117.83	111.73
5	C	101	LFI	C2-N2-C6	2.43	130.37	122.49

There are no chirality outliers.

5 of 24 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
5	C	101	LFI	C8-C4-N1-C3
5	C	101	LFI	O2-C4-N1-C3
5	C	101	LFI	C9-C5-N3-C2
5	C	101	LFI	C9-C5-N3-C3
5	C	101	LFI	O3-C5-N3-C2

There are no ring outliers.

2 monomers are involved in 3 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
5	C	101	LFI	1	0
5	D	101	LFI	2	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 LFI C 101

Bond lengths | Bond angles

Torsions | Rings

Ligand LFI D 101
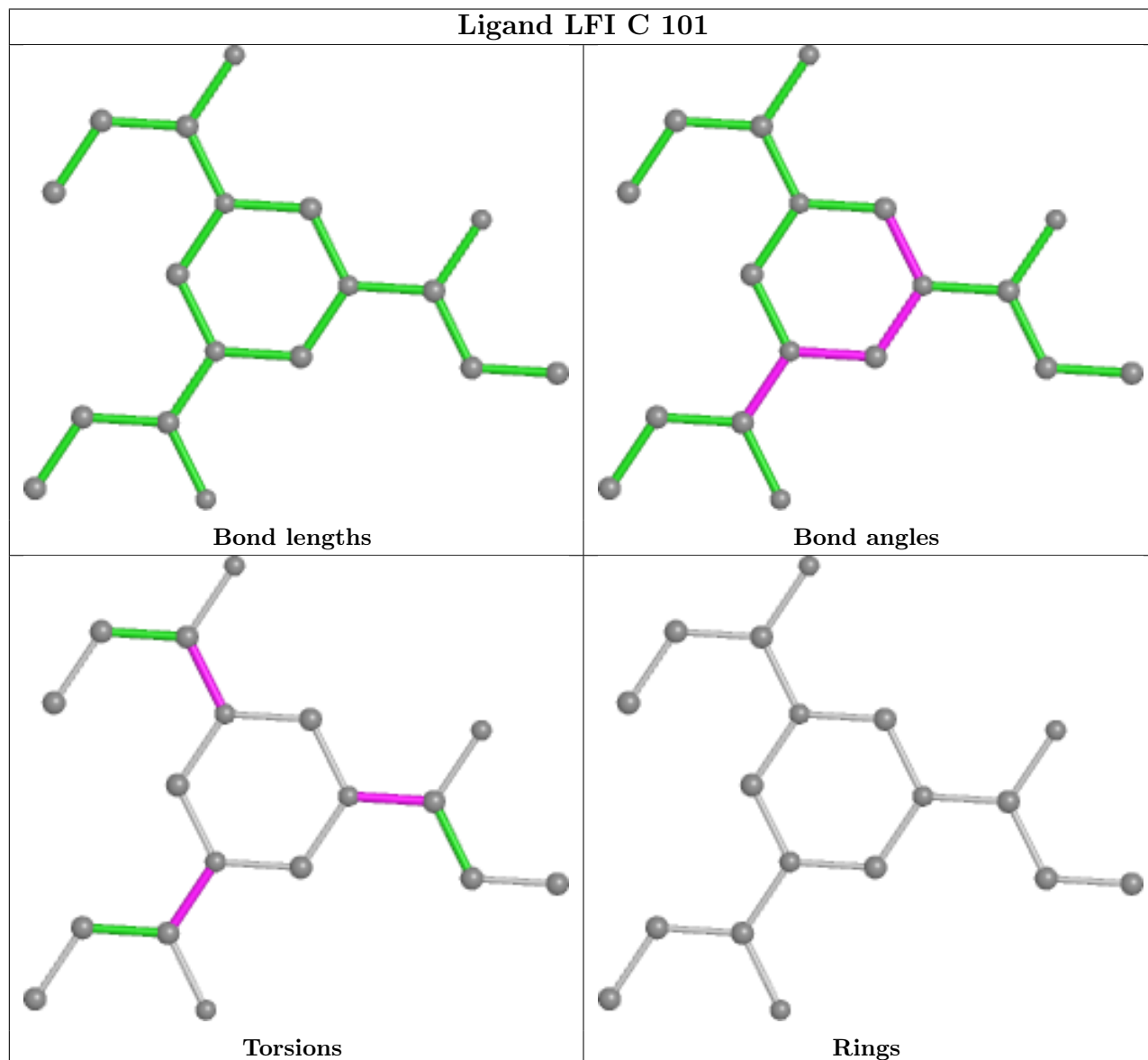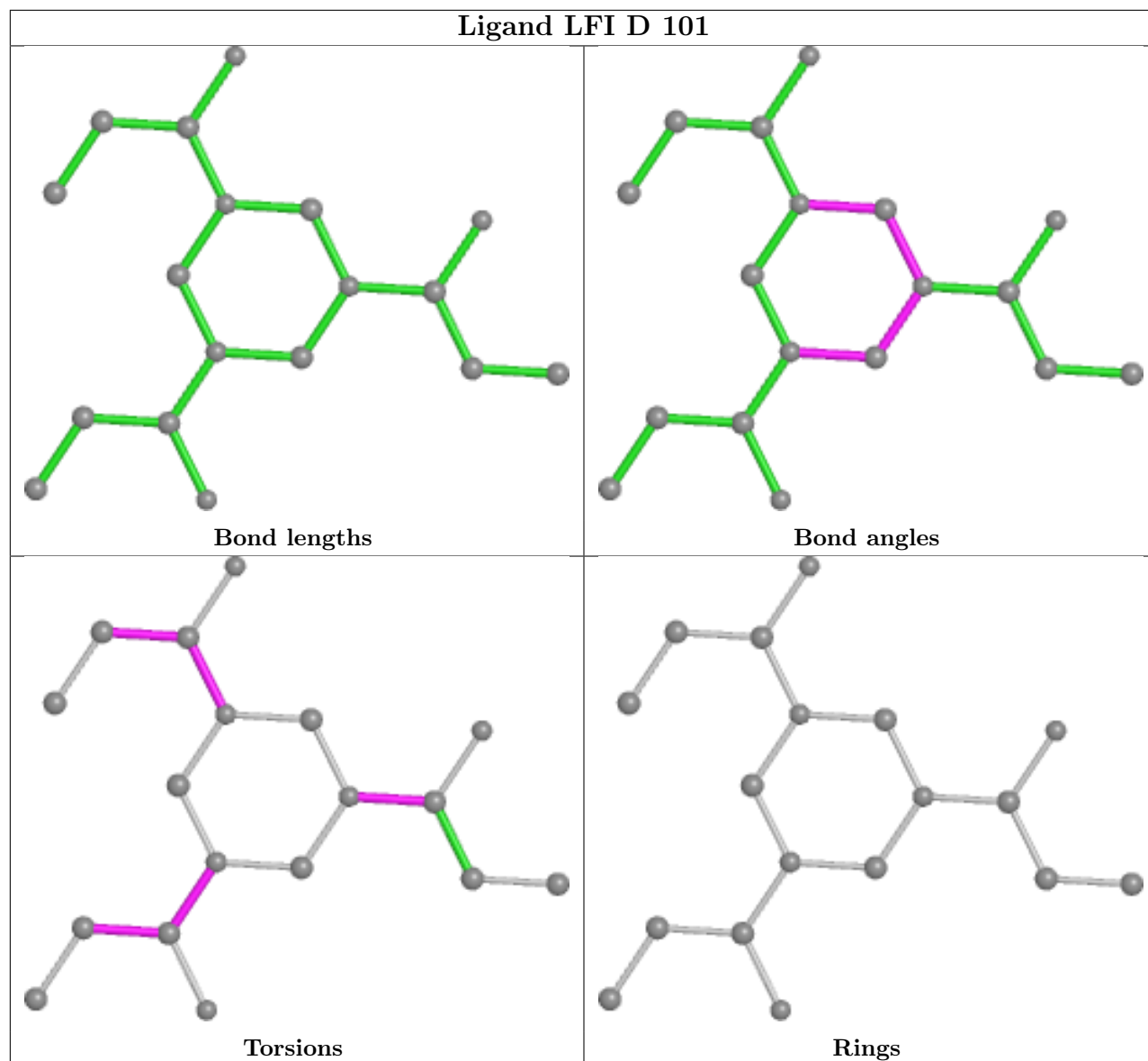
5.7 Other polymers ⓘ

There are no such residues in this entry.

5.8 Polymer linkage issues ⓘ

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	596/609 (97%)	0.18	14 (2%)	60	63	40, 64, 103, 158	0
1	B	595/609 (97%)	0.53	42 (7%)	16	16	45, 91, 136, 161	0
2	C	17/18 (94%)	1.41	2 (11%)	4	4	72, 90, 141, 144	0
2	D	17/18 (94%)	0.85	1 (5%)	22	23	56, 67, 109, 124	0
All	All	1225/1254 (97%)	0.38	59 (4%)	30	31	40, 72, 131, 161	0

The worst 5 of 59 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
2	C	16	ALA	12.3
1	B	342	ALA	6.3
1	B	103	ASN	5.7
1	A	338	ASN	5.7
1	B	574	VAL	5.6

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

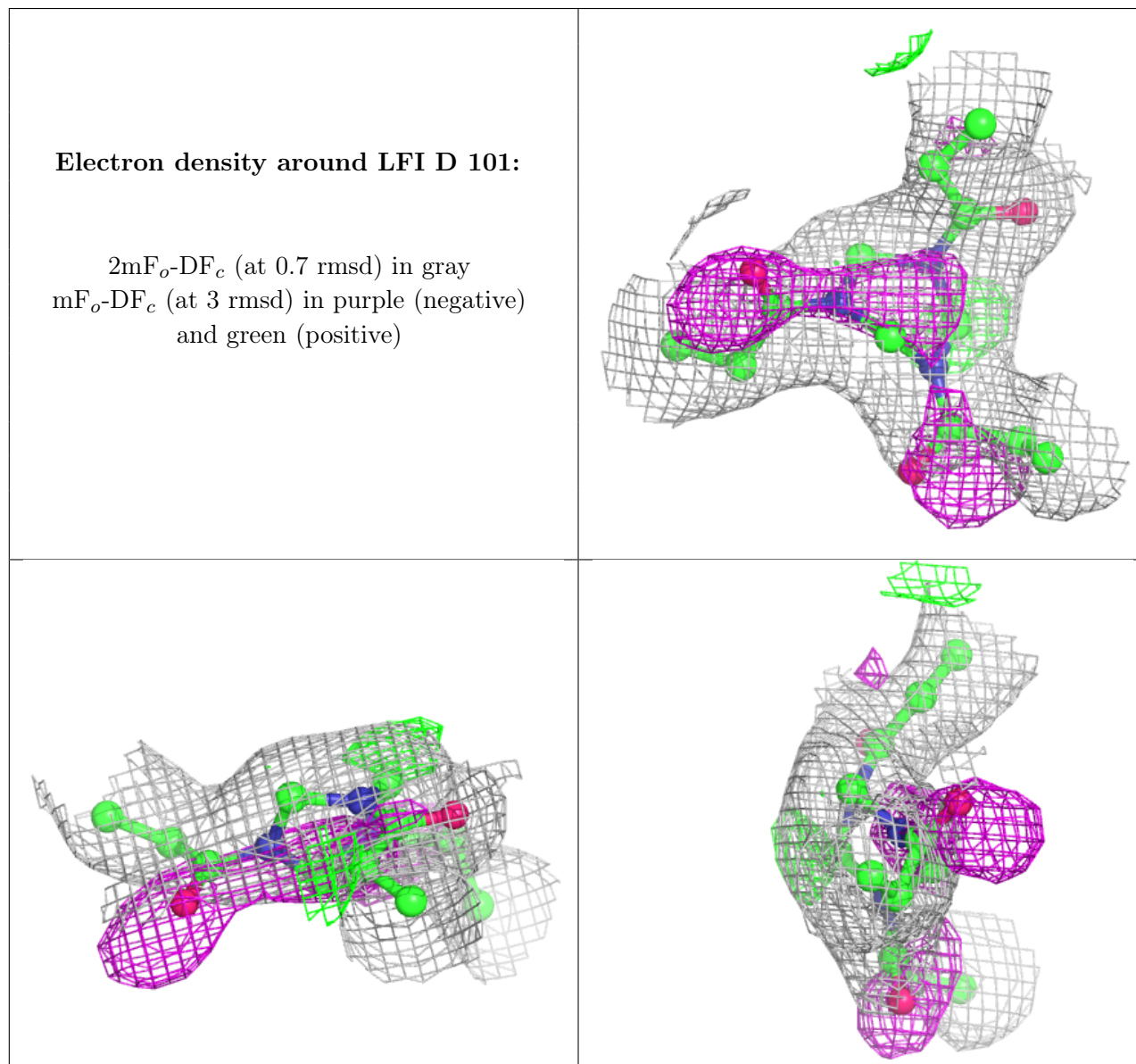There are no non-standard protein/DNA/RNA residues in this entry.

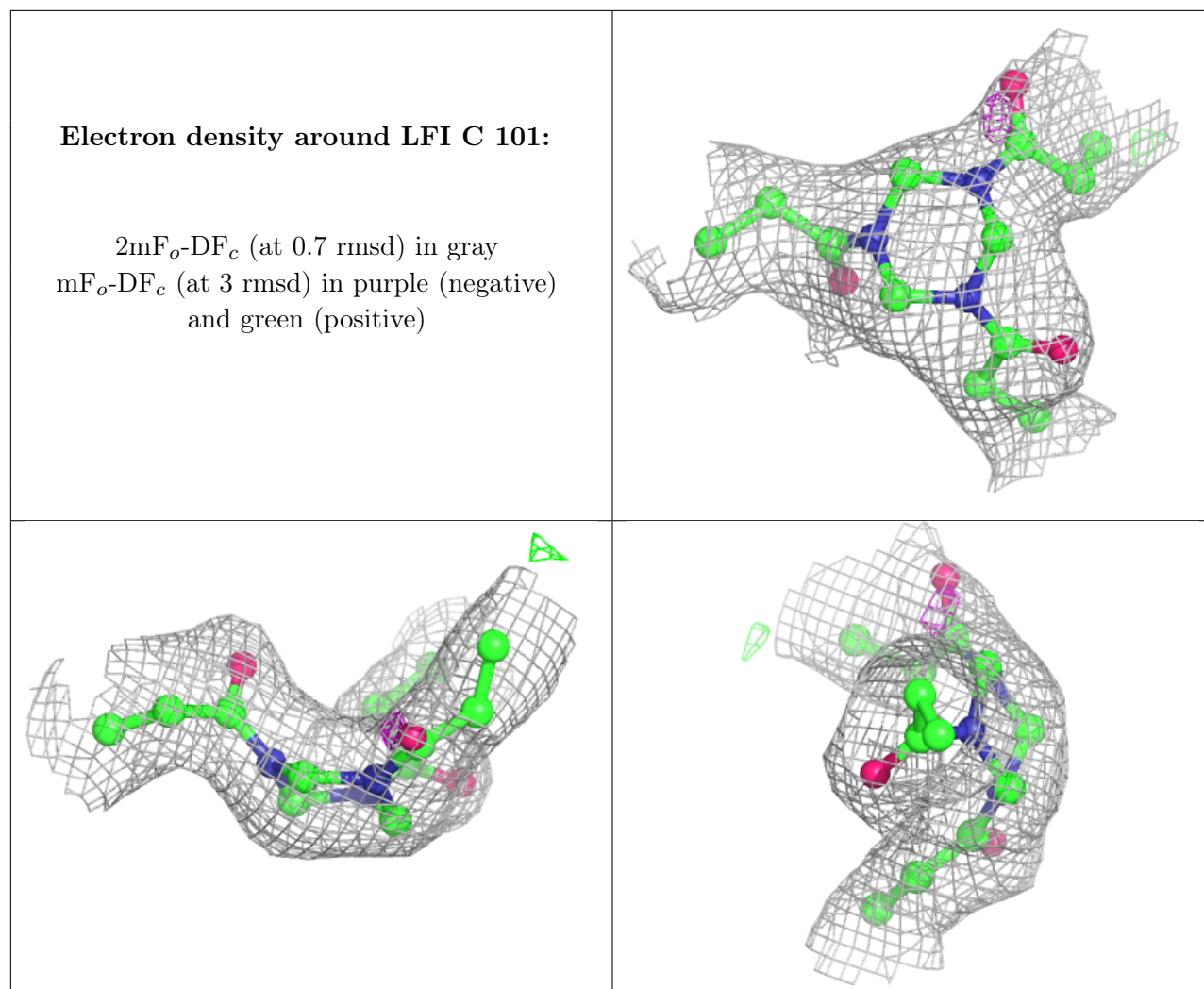## 6.3 Carbohydrates ⓘ

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
5	LFI	D	101	18/21	0.73	0.20	46,56,72,75	0
4	NA	B	702	1/1	0.78	0.15	75,75,75,75	0
5	LFI	C	101	18/21	0.89	0.10	56,64,77,80	0
3	ZN	B	701	1/1	0.96	0.15	82,82,82,82	0
3	ZN	A	701	1/1	0.99	0.17	48,48,48,48	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 LFI D 101:

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

Electron density around LFI C 101:

$2mF_o$-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.