WORLDWIDE PDB PROTEIN DATA BANK

wwPDB X-ray Structure Validation Summary Report (i)

Nov 30, 2022 – 12:28 pm GMT

PDB ID	:	6QUJ
Title	:	GHK tagged GFP variant
Authors	:	Huyton, T.; Gorlich, D.
Deposited on	:	2019-02-27
Resolution	:	1.68 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	:	2.31.3
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0267
CCP4	:	7.1.010 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.31.3

1 Overall quality at a glance ⓘ

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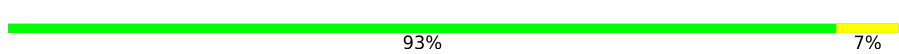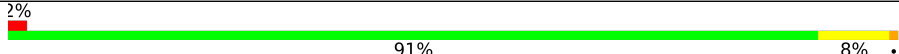*X-RAY DIFFRACTION*

The reported resolution of this entry is 1.68 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.
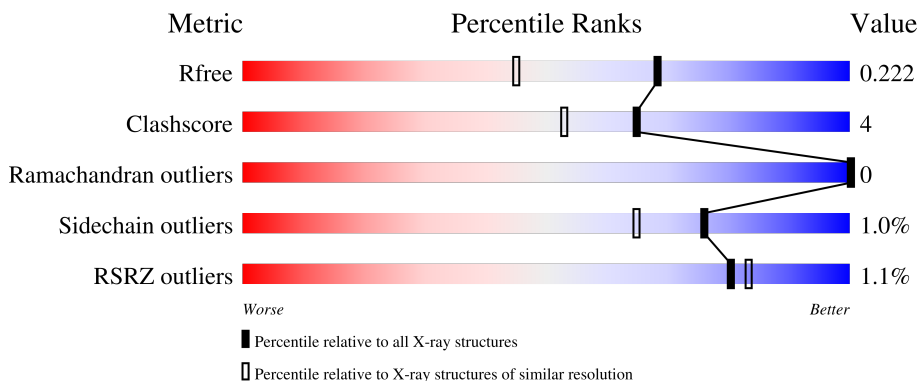
Metric	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
R_{free}	130704	6780 (1.70-1.66)
Clashscore	141614	7310 (1.70-1.66)
Ramachandran outliers	138981	7173 (1.70-1.66)
Sidechain outliers	138945	7172 (1.70-1.66)
RSRZ outliers	127900	6661 (1.70-1.66)

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	B	229	93% · 7%
1	E	229	2% · · · 91% · 8% ·

2 Entry composition (i)

There are 5 unique types of molecules in this entry. The entry contains 4030 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 Green fluorescent protein.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	B	229	Total 1868	C 1158	N 357	O 348	S 5	0	1	0
1	E	229	Total 1864	C 1156	N 354	O 349	S 5	0	1	0

There are 82 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	0	GLY	-	expression tag	UNP P42212
B	1	HIS	SER	conflict	UNP P42212
B	3	ARG	-	insertion	UNP P42212
B	6	GLN	GLU	conflict	UNP P42212
B	26	ARG	LYS	conflict	UNP P42212
B	30	ARG	SER	conflict	UNP P42212
B	39	ASN	TYR	conflict	UNP P42212
B	41	ARG	LYS	conflict	UNP P42212
B	45	ARG	LYS	conflict	UNP P42212
B	52	ARG	LYS	conflict	UNP P42212
B	?	-	PHE	deletion	UNP P42212
B	?	-	SER	deletion	UNP P42212
B	64	LEU	TYR	conflict	UNP P42212
B	65	CRO	GLY	conflict	UNP P42212
B	77	ARG	LYS	conflict	UNP P42212
B	78	ARG	GLN	conflict	UNP P42212
B	97	SER	PHE	conflict	UNP P42212
B	99	ARG	LYS	conflict	UNP P42212
B	103	THR	ASN	conflict	UNP P42212
B	105	ARG	LYS	conflict	UNP P42212
B	109	VAL	GLU	conflict	UNP P42212
B	111	ARG	LYS	conflict	UNP P42212
B	115	ASN	ASP	conflict	UNP P42212
B	124	ARG	LYS	conflict	UNP P42212
B	126	THR	ILE	conflict	UNP P42212

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Chain	Residue	Modelled	Actual	Comment	Reference
B	127	ASN	ASP	conflict	UNP P42212
B	129	ARG	LYS	conflict	UNP P42212
B	138	ARG	LYS	conflict	UNP P42212
B	151	THR	MET	conflict	UNP P42212
B	154	ARG	LYS	conflict	UNP P42212
B	156	ARG	LYS	conflict	UNP P42212
B	160	ARG	LYS	conflict	UNP P42212
B	161	ALA	VAL	conflict	UNP P42212
B	164	THR	LYS	conflict	UNP P42212
B	169	VAL	ILE	conflict	UNP P42212
B	178	ASN	ASP	conflict	UNP P42212
B	188	ASN	ASP	conflict	UNP P42212
B	196	ASP	ASN	conflict	UNP P42212
B	203	THR	SER	conflict	UNP P42212
B	207	ARG	LYS	conflict	UNP P42212
B	212	ARG	LYS	conflict	UNP P42212
E	0	GLY	-	expression tag	UNP P42212
E	1	HIS	SER	conflict	UNP P42212
E	3	ARG	-	insertion	UNP P42212
E	6	GLN	GLU	conflict	UNP P42212
E	26	ARG	LYS	conflict	UNP P42212
E	30	ARG	SER	conflict	UNP P42212
E	39	ASN	TYR	conflict	UNP P42212
E	41	ARG	LYS	conflict	UNP P42212
E	45	ARG	LYS	conflict	UNP P42212
E	52	ARG	LYS	conflict	UNP P42212
E	?	-	PHE	deletion	UNP P42212
E	?	-	SER	deletion	UNP P42212
E	64	LEU	TYR	conflict	UNP P42212
E	65	CRO	GLY	conflict	UNP P42212
E	77	ARG	LYS	conflict	UNP P42212
E	78	ARG	GLN	conflict	UNP P42212
E	97	SER	PHE	conflict	UNP P42212
E	99	ARG	LYS	conflict	UNP P42212
E	103	THR	ASN	conflict	UNP P42212
E	105	ARG	LYS	conflict	UNP P42212
E	109	VAL	GLU	conflict	UNP P42212
E	111	ARG	LYS	conflict	UNP P42212
E	115	ASN	ASP	conflict	UNP P42212
E	124	ARG	LYS	conflict	UNP P42212
E	126	THR	ILE	conflict	UNP P42212
E	127	ASN	ASP	conflict	UNP P42212

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Chain	Residue	Modelled	Actual	Comment	Reference
E	129	ARG	LYS	conflict	UNP P42212
E	138	ARG	LYS	conflict	UNP P42212
E	151	THR	MET	conflict	UNP P42212
E	154	ARG	LYS	conflict	UNP P42212
E	156	ARG	LYS	conflict	UNP P42212
E	160	ARG	LYS	conflict	UNP P42212
E	161	ALA	VAL	conflict	UNP P42212
E	164	THR	LYS	conflict	UNP P42212
E	169	VAL	ILE	conflict	UNP P42212
E	178	ASN	ASP	conflict	UNP P42212
E	188	ASN	ASP	conflict	UNP P42212
E	196	ASP	ASN	conflict	UNP P42212
E	203	THR	SER	conflict	UNP P42212
E	207	ARG	LYS	conflict	UNP P42212
E	212	ARG	LYS	conflict	UNP P42212

- Molecule 2 is COPPER (II) ION (three-letter code: CU) (formula: Cu) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
2	B	1	Total 1	Cu 1	0	0
2	E	1	Total 1	Cu 1	0	0

- Molecule 3 is SULFATE ION (three-letter code: SO4) (formula: O_4S).
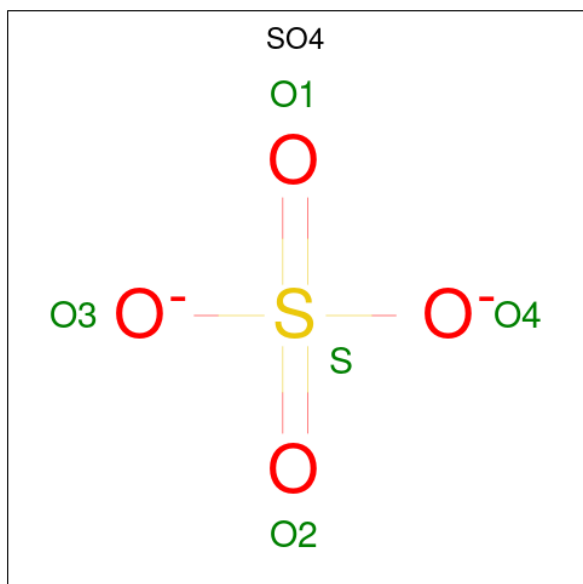
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
3	B	1	Total 5	O 4	S 1	0	0
3	B	1	Total 5	O 4	S 1	0	0
3	E	1	Total 5	O 4	S 1	0	0

- Molecule 4 is GLYCEROL (three-letter code: GOL) (formula: $C_3H_8O_3$).

GOL
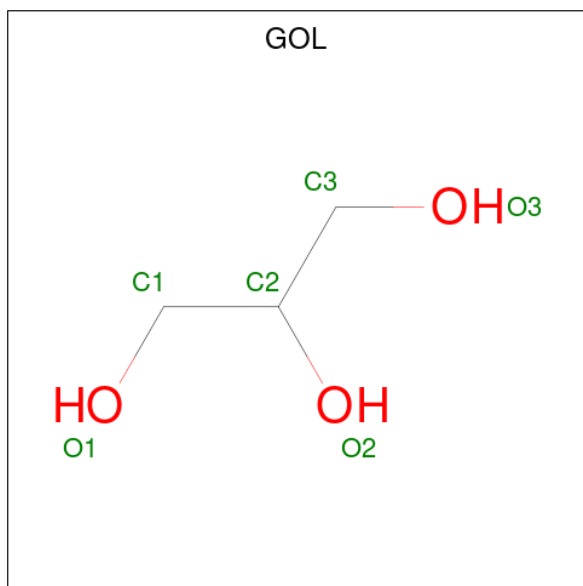
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
4	E	1	Total 6	C 3	O 3	0	0
4	E	1	Total 6	C 3	O 3	0	0

- Molecule 5 is water.

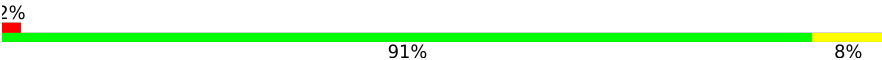| Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
5	B	149	Total 149	O 149	0	0
5	E	120	Total 120	O 120	0	0

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.
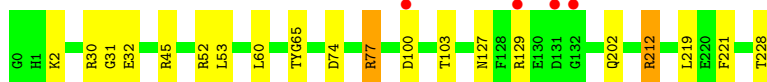
• Molecule 1: Green fluorescent protein

Chain B:

• Molecule 1: Green fluorescent protein

Chain E:

4 Data and refinement statistics ⓘ

Property	Value	Source
Space group	P 65 2 2	Depositor
Cell constants a, b, c, α, β, γ	85.99Å 85.99Å 270.14Å 90.00° 90.00° 120.00°	Depositor
Resolution (Å)	50.08 – 1.68 50.03 – 1.68	Depositor EDS
% Data completeness (in resolution range)	100.0 (50.08-1.68) 100.0 (50.03-1.68)	Depositor EDS
R_{merge}	0.06	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	1.54 (at 1.68Å)	Xtriage
Refinement program	REFMAC 5.8.0253	Depositor
R, R_{free}	0.172 , 0.221 0.170 , 0.222	Depositor DCC
R_{free} test set	3473 reflections (5.08%)	wwPDB-VP
Wilson B-factor (Å^2)	23.8	Xtriage
Anisotropy	1.437	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	(Not available) , (Not available)	EDS
L-test for twinning[2]	$< |L| > = 0.49, < L^2 > = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.98	EDS
Total number of atoms	4030	wwPDB-VP
Average B, all atoms (Å^2)	44.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The analyses of the Patterson function reveals a significant off-origin peak that is 44.59 % of the origin peak, indicating pseudo-translational symmetry. The chance of finding a peak of this or larger height randomly in a structure without pseudo-translational symmetry is equal to 1.4925e-04. The detected translational NCS is most likely also responsible for the elevated intensity ratio.*

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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: CU, GOL, SO4, CRO

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	B	0.72	0/1885	0.82	0/2552
1	E	0.71	0/1881	0.80	0/2548
All	All	0.72	0/3766	0.81	0/5100

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 ⓘ

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	B	1868	0	1797	17	0
1	E	1864	0	1792	14	0
2	B	1	0	0	0	0
2	E	1	0	0	0	0
3	B	10	0	0	0	0
3	E	5	0	0	0	0
4	E	12	0	16	2	0
5	B	149	0	0	0	0
5	E	120	0	0	1	0
All	All	4030	0	3605	30	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.

The worst 5 of 30 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:69:PHE:CE2	1:B:117:LEU:HD23	1.67	1.28
1:B:69:PHE:CE2	1:B:117:LEU:CD2	2.34	1.10
1:E:74:ASP:O	1:E:77:ARG:HG2	1.52	1.10
1:B:69:PHE:HE2	1:B:117:LEU:HD23	0.95	1.06
1:B:69:PHE:HE2	1:B:117:LEU:CD2	1.80	0.85

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	B	225/229 (98%)	223 (99%)	2 (1%)	0	100	100
1	E	225/229 (98%)	223 (99%)	2 (1%)	0	100	100
All	All	450/458 (98%)	446 (99%)	4 (1%)	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	B	199/198 (100%)	198 (100%)	1 (0%)	88	83
1	E	199/198 (100%)	196 (98%)	3 (2%)	65	48
All	All	398/396 (100%)	394 (99%)	4 (1%)	76	65

All (4) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	99	ARG
1	E	52	ARG
1	E	77	ARG
1	E	212	ARG

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (2) such sidechains are listed below:

Mol	Chain	Res	Type
1	B	178	ASN
1	E	157	ASN

5.3.3   RNA ⓘ

There are no RNA molecules in this entry.

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

2 non-standard protein/DNA/RNA residues 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	#$|Z| > 2$
1	CRO	E	65	1	23,23,24	2.84	7 (30%)	30,32,34	2.25	9 (30%)
1	CRO	B	65	1	23,23,24	2.86	7 (30%)	30,32,34	2.52	9 (30%)

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
1	CRO	E	65	1	-	2/12/31/32	0/2/2/2
1	CRO	B	65	1	-	2/12/31/32	0/2/2/2

The worst 5 of 14 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	B	65	CRO	CB2-CA2	-7.37	1.29	1.35
1	E	65	CRO	CB2-CA2	-6.81	1.29	1.35
1	B	65	CRO	CA2-C2	6.51	1.55	1.48
1	E	65	CRO	C1-N2	5.98	1.41	1.32
1	E	65	CRO	CA2-C2	5.79	1.54	1.48

The worst 5 of 18 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	B	65	CRO	CA2-C2-N3	7.75	107.04	103.37
1	E	65	CRO	CA2-C2-N3	6.02	106.22	103.37
1	B	65	CRO	O3-C3-CA3	-5.32	110.31	126.39
1	E	65	CRO	O3-C3-CA3	-4.84	111.77	126.39
1	B	65	CRO	C2-N3-C1	-4.64	105.62	107.97

There are no chirality outliers.

All (4) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	E	65	CRO	N2-CA2-CB2-CG2
1	B	65	CRO	N2-CA2-CB2-CG2
1	E	65	CRO	C2-CA2-CB2-CG2
1	B	65	CRO	C2-CA2-CB2-CG2

There are no ring outliers.

No monomer is involved in short contacts.

5.5 Carbohydrates ⓘ

There are no monosaccharides in this entry.

5.6 Ligand geometry ⓘ

Of 7 ligands modelled in this entry, 2 are monoatomic - leaving 5 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	$\#|Z| > 2$
3	SO4	B	303	-	4,4,4	0.51	0	6,6,6	0.16	0
4	GOL	E	301	-	5,5,5	0.11	0	5,5,5	0.36	0
3	SO4	B	302	-	4,4,4	0.41	0	6,6,6	0.08	0
4	GOL	E	302	-	5,5,5	0.15	0	5,5,5	0.51	0
3	SO4	E	304	-	4,4,4	0.41	0	6,6,6	0.14	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
4	GOL	E	302	-	-	0/4/4/4	-
4	GOL	E	301	-	-	2/4/4/4	-

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (2) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
4	E	301	GOL	C1-C2-C3-O3
4	E	301	GOL	O2-C2-C3-O3

There are no ring outliers.

1 monomer is involved in 2 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	E	301	GOL	2	0

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	B	228/229 (99%)	0.08	1 (0%)	92	93	28, 39, 62, 82	1 (0%)
1	E	228/229 (99%)	0.12	4 (1%)	68	72	30, 42, 64, 92	3 (1%)
All	All	456/458 (99%)	0.10	5 (1%)	80	83	28, 40, 65, 92	4 (0%)

All (5) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	E	131	ASP	4.1
1	E	132	GLY	3.0
1	E	100	ASP	2.7
1	E	129	ARG	2.2
1	B	131	ASP	2.2

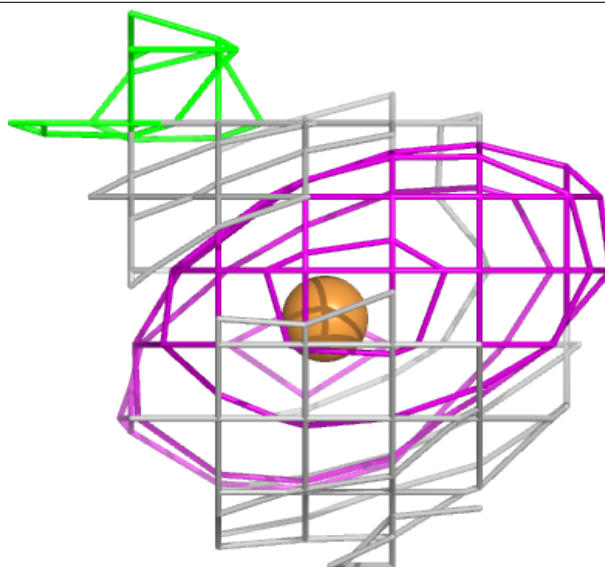## 6.2 Non-standard residues in protein, DNA, RNA chains ⓘ

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
1	CRO	B	65	22/23	0.96	0.10	27,31,33,34	0
1	CRO	E	65	22/23	0.96	0.09	30,32,36,39	0

6.3 Carbohydrates ⓘ

There are no monosaccharides in this entry.

WORLDWIDE
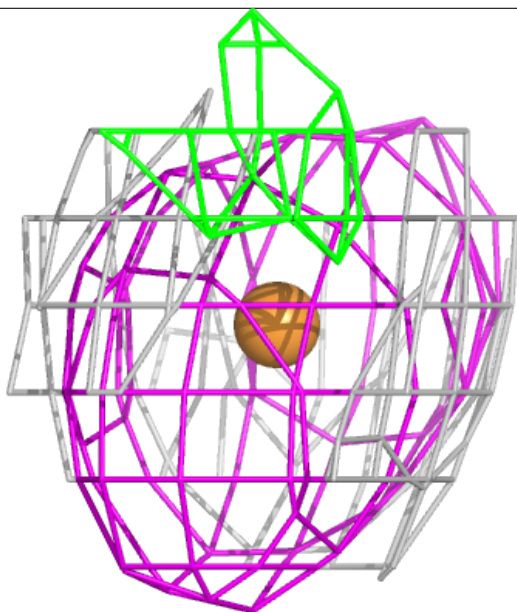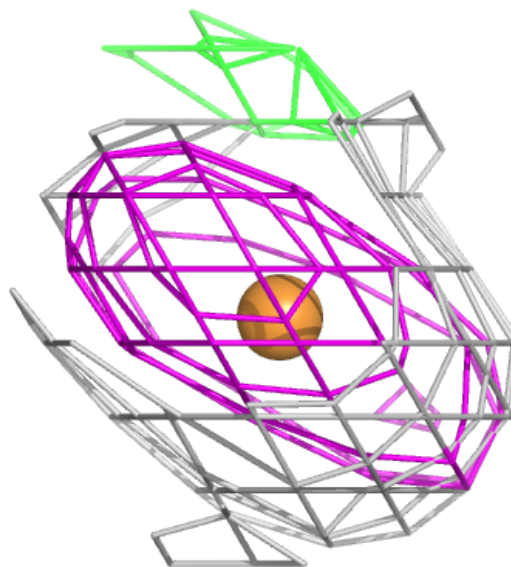wwPDB
PROTEIN DATA BANK

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.
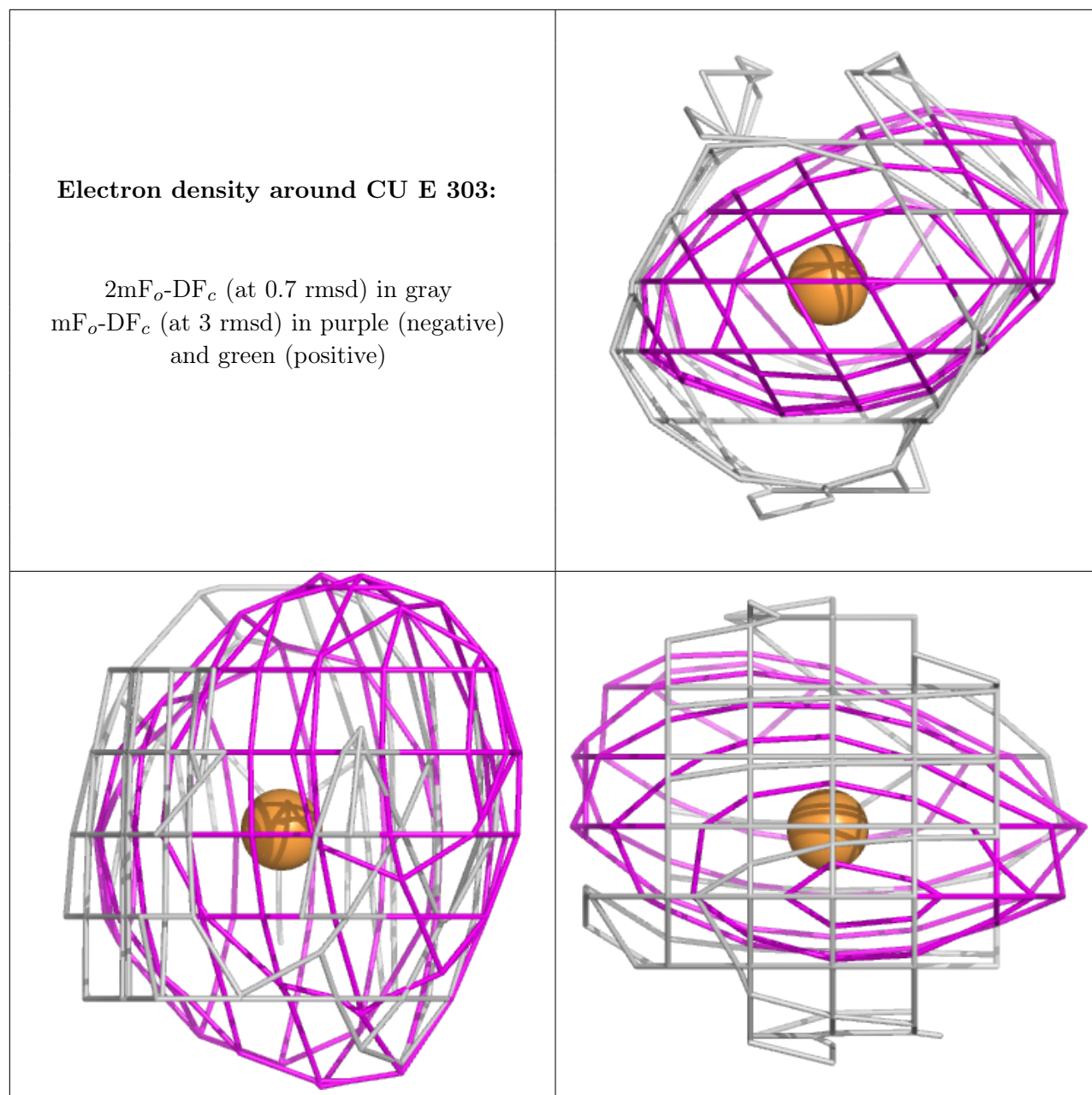
Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å^2)	Q<0.9
4	GOL	E	301	6/6	0.69	0.24	68,71,76,77	0
4	GOL	E	302	6/6	0.86	0.18	57,71,85,90	0
3	SO4	B	302	5/5	0.96	0.11	62,73,79,83	1
3	SO4	E	304	5/5	0.96	0.21	48,51,62,73	1
3	SO4	B	303	5/5	0.98	0.09	38,44,47,48	1
2	CU	B	301	1/1	0.99	0.04	57,57,57,57	0
2	CU	E	303	1/1	1.00	0.04	41,41,41,41	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 CU B 301:

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

Electron density around CU E 303:

$2mF_o$-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.