



# wwPDB X-ray Structure Validation Summary Report

Sep 4, 2024 – 08:10 PM JST

PDB ID : 8WGM  
Title : Quadruple mutant Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS V1/S, N51I+C59R+S108N+I164L) complexed with LA1, NADPH and dUMP  
Authors : Vanichtanankul, J.; Saeyang, T.; Vitsupakorn, D.; Arwon, U.; Decharuangsilp, S.; Yuthavong, Y.; Kamchonwongpaisan, S.  
Deposited on : 2023-09-22  
Resolution : 2.15 Å(reported)

This is a wwPDB X-ray Structure Validation Summary Report for a publicly released PDB entry.

We welcome your comments at [validation@mail.wwpdb.org](mailto: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>.

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The following versions of software and data (see [references](#) ①) were used in the production of this report:

MolProbity : 4.02b-467  
Mogul : 1.8.5 (274361), CSD as541be (2020)  
Xtrriage (Phenix) : 1.13  
EDS : 3.0  
buster-report : 1.1.7 (2018)  
Percentile statistics : 20231227.v01 (using entries in the PDB archive December 27th 2023)  
CCP4 : 9.0.002 (Gargrove)  
Density-Fitness : 1.0.11  
Ideal geometry (proteins) : Engh & Huber (2001)  
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)  
Validation Pipeline (wwPDB-VP) : 2.38.2

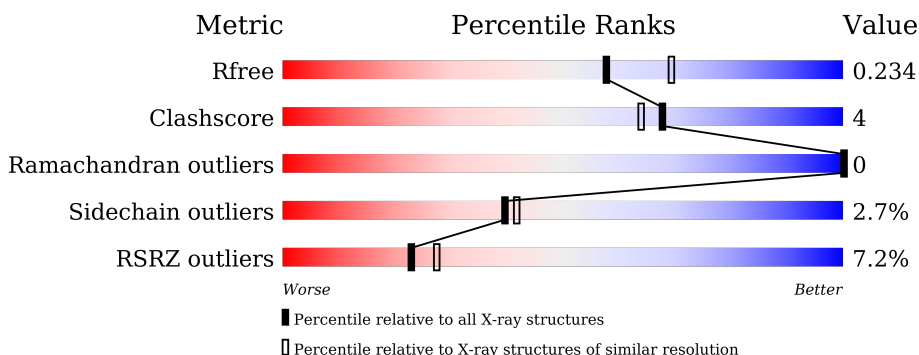
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*X-RAY DIFFRACTION*

The reported resolution of this entry is 2.15 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))
$R_{free}$	164625	1881 (2.16-2.16)
Clashscore	180529	2047 (2.16-2.16)
Ramachandran outliers	177936	2027 (2.16-2.16)
Sidechain outliers	177891	2026 (2.16-2.16)
RSRZ outliers	164620	1882 (2.16-2.16)

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  $\geq 3$ , 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	608	 4% 79% 7% • 12%
1	B	608	 8% 76% 10% • 13%

## 2 Entry composition

There are 5 unique types of molecules in this entry. The entry contains 9780 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.

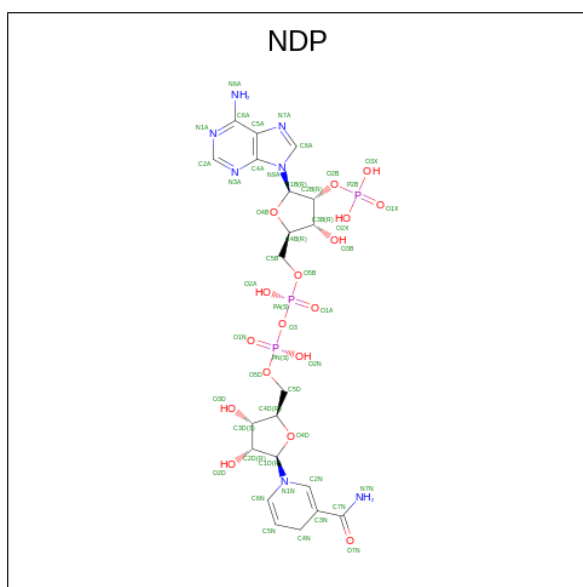
- Molecule 1 is a protein called Bifunctional dihydrofolate reductase-thymidylate synthase.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	532	4422	2863	729	805	25	0	0	0
1	B	528	4392	2845	725	798	24	0	0	0

There are 14 discrepancies between the modelled and reference sequences:

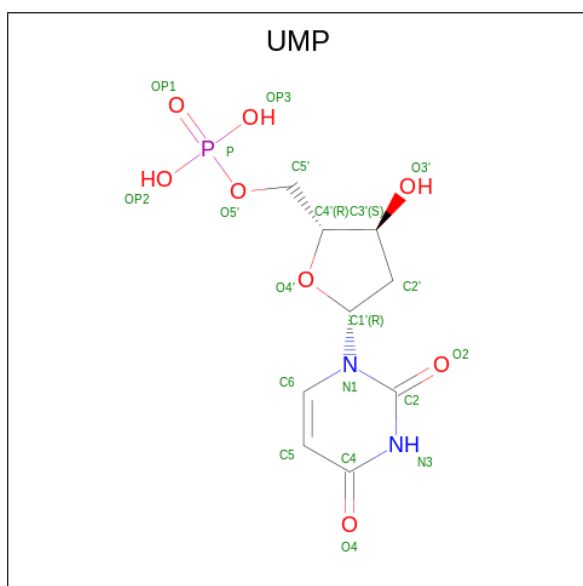
Chain	Residue	Modelled	Actual	Comment	Reference
A	26	ALA	GLY	conflict	UNP A7UD81
A	27	ALA	LYS	conflict	UNP A7UD81
A	28	ALA	LYS	conflict	UNP A7UD81
A	51	ILE	ASN	engineered mutation	UNP A7UD81
A	59	ARG	CYS	engineered mutation	UNP A7UD81
A	108	ASN	SER	engineered mutation	UNP A7UD81
A	164	LEU	ILE	engineered mutation	UNP A7UD81
B	26	ALA	GLY	conflict	UNP A7UD81
B	27	ALA	LYS	conflict	UNP A7UD81
B	28	ALA	LYS	conflict	UNP A7UD81
B	51	ILE	ASN	engineered mutation	UNP A7UD81
B	59	ARG	CYS	engineered mutation	UNP A7UD81
B	108	ASN	SER	engineered mutation	UNP A7UD81
B	164	LEU	ILE	engineered mutation	UNP A7UD81

- Molecule 2 is NADPH DIHYDRO-NICOTINAMIDE-ADENINE-DINUCLEOTIDE PHOSPHATE (three-letter code: NDP) (formula: C<sub>21</sub>H<sub>30</sub>N<sub>7</sub>O<sub>17</sub>P<sub>3</sub>).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	
			Total	C	N	O			P
2	A	1	48	21	7	17	3	0	0
2	B	1	48	21	7	17	3	0	0

- Molecule 3 is 2'-DEOXYURIDINE 5'-MONOPHOSPHATE (three-letter code: UMP) (formula:  $C_9H_{13}N_2O_8P$ ).



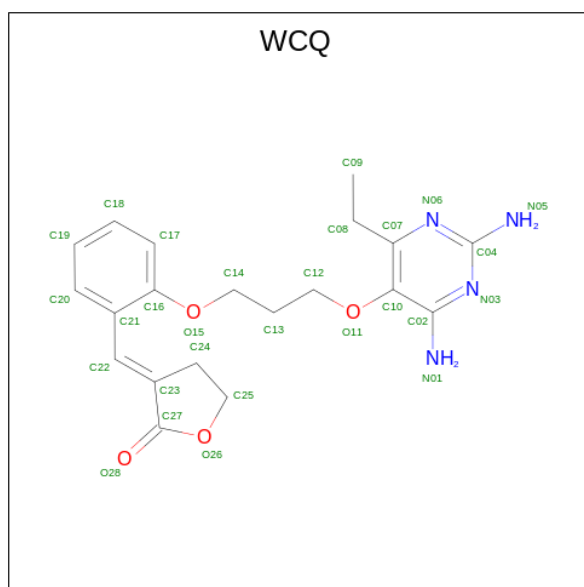
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	
			Total	C	N	O			P
3	A	1	20	9	2	8	1	0	0

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Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
3	B	1	Total	C	N	O	P	0	0
			20	9	2	8	1		

- Molecule 4 is (3 {E})-3-[[2-[3-[2,4-bis(azanyl)-6-ethyl-pyrimidin-5-yl]oxypropoxy]phenyl]methylidene]oxolan-2-one (three-letter code: WCQ) (formula: C<sub>20</sub>H<sub>24</sub>N<sub>4</sub>O<sub>4</sub>) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
4	A	1	Total	C	N	O	0	0
			28	20	4	4		
4	B	1	Total	C	N	O	0	0
			28	20	4	4		

- Molecule 5 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
5	A	421	Total	O	0	0
			421	421		
5	B	353	Total	O	0	0
			353	353		



## 4 Data and refinement statistics

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	58.35Å 156.28Å 165.00Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	24.32 – 2.15 24.32 – 2.15	Depositor EDS
% Data completeness (in resolution range)	99.2 (24.32-2.15) 89.1 (24.32-2.15)	Depositor EDS
$R_{merge}$	(Not available)	Depositor
$R_{sym}$	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	1.30 (at 2.15Å)	Xtrriage
Refinement program	PHENIX 1.20.1_4487	Depositor
R, $R_{free}$	0.190 , 0.233 0.190 , 0.234	Depositor DCC
$R_{free}$ test set	80955 reflections (2.40%)	wwPDB-VP
Wilson B-factor (Å <sup>2</sup> )	20.9	Xtrriage
Anisotropy	0.360	Xtrriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.38 , 57.4	EDS
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.47$ , $\langle L^2 \rangle = 0.30$	Xtrriage
Estimated twinning fraction	No twinning to report.	Xtrriage
$F_o, F_c$ correlation	0.94	EDS
Total number of atoms	9780	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	34.0	wwPDB-VP

Xtrriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.49% of the height of the origin peak. No significant pseudotranslation is detected.*

<sup>1</sup>Intensities estimated from amplitudes.

<sup>2</sup>Theoretical 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: NDP, WCQ, UMP

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	0/4525	0.60	0/6111
1	B	0.43	1/4495 (0.0%)	0.58	0/6073
All	All	0.43	1/9020 (0.0%)	0.59	0/12184

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	B	494	CYS	CB-SG	-7.13	1.70	1.82

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	4422	0	4379	31	0
1	B	4392	0	4347	34	0
2	A	48	0	26	1	0
2	B	48	0	26	2	0
3	A	20	0	11	0	0
3	B	20	0	11	0	0
4	A	28	0	0	0	0
4	B	28	0	0	0	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
5	A	421	0	0	4	0
5	B	353	0	0	6	0
All	All	9780	0	8800	63	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 63 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:119:LEU:H	1:A:124:ASN:HD21	1.27	0.81
1:B:8:VAL:HA	1:B:76:LYS:HD3	1.68	0.76
1:A:491:HIS:HD2	5:A:979:HOH:O	1.77	0.68
1:A:201:ASN:HB3	1:A:204:GLU:HB2	1.81	0.63
1:A:373:LYS:HB3	1:A:601:LYS:HB3	1.84	0.60

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	522/608 (86%)	509 (98%)	13 (2%)	0	100	100
1	B	518/608 (85%)	502 (97%)	16 (3%)	0	100	100
All	All	1040/1216 (86%)	1011 (97%)	29 (3%)	0	100	100

There are no Ramachandran outliers to report.

### 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	495/568 (87%)	484 (98%)	11 (2%)	47	51
1	B	493/568 (87%)	477 (97%)	16 (3%)	34	34
All	All	988/1136 (87%)	961 (97%)	27 (3%)	40	42

5 of 27 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	87	ASP
1	B	140	VAL
1	B	346	THR
1	B	135	ASP
1	B	144	ASN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 12 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	294	ASN
1	B	407	ASN
1	B	554	ASN
1	B	450	ASN
1	A	415	ASN

### 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 [i](#)

6 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
2	NDP	A	701	-	45,52,52	2.21	6 (13%)	53,80,80	1.51	8 (15%)
3	UMP	B	702	-	21,21,21	0.91	0	31,31,31	1.40	6 (19%)
4	WCQ	B	703	-	29,30,30	2.35	6 (20%)	36,40,40	2.04	10 (27%)
3	UMP	A	702	-	21,21,21	0.87	0	31,31,31	1.61	7 (22%)
4	WCQ	A	703	-	29,30,30	2.29	7 (24%)	36,40,40	2.30	11 (30%)
2	NDP	B	701	-	45,52,52	2.13	6 (13%)	53,80,80	1.57	8 (15%)

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	NDP	A	701	-	-	5/30/77/77	0/5/5/5
3	UMP	B	702	-	-	2/10/22/22	0/2/2/2
4	WCQ	B	703	-	-	0/14/24/24	0/3/3/3
3	UMP	A	702	-	-	2/10/22/22	0/2/2/2
4	WCQ	A	703	-	-	0/14/24/24	0/3/3/3
2	NDP	B	701	-	-	4/30/77/77	0/5/5/5

The worst 5 of 25 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	701	NDP	P2B-O2B	11.77	1.81	1.59

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Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	B	701	NDP	P2B-O2B	11.22	1.80	1.59
4	B	703	WCQ	O26-C27	6.34	1.46	1.36
4	A	703	WCQ	O26-C27	6.06	1.46	1.36
4	A	703	WCQ	C04-N05	5.61	1.45	1.33

The worst 5 of 50 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
4	A	703	WCQ	C04-N06-C07	7.91	122.84	116.24
4	B	703	WCQ	C04-N06-C07	5.77	121.05	116.24
4	A	703	WCQ	C25-O26-C27	-5.45	106.11	110.74
2	B	701	NDP	PN-O3-PA	-5.29	114.67	132.83
2	A	701	NDP	PN-O3-PA	-5.02	115.61	132.83

There are no chirality outliers.

5 of 13 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	B	702	UMP	C3'-C4'-C5'-O5'
3	B	702	UMP	O4'-C4'-C5'-O5'
2	A	701	NDP	PA-O3-PN-O5D
2	B	701	NDP	C2D-C1D-N1N-C2N
2	B	701	NDP	O4D-C1D-N1N-C2N

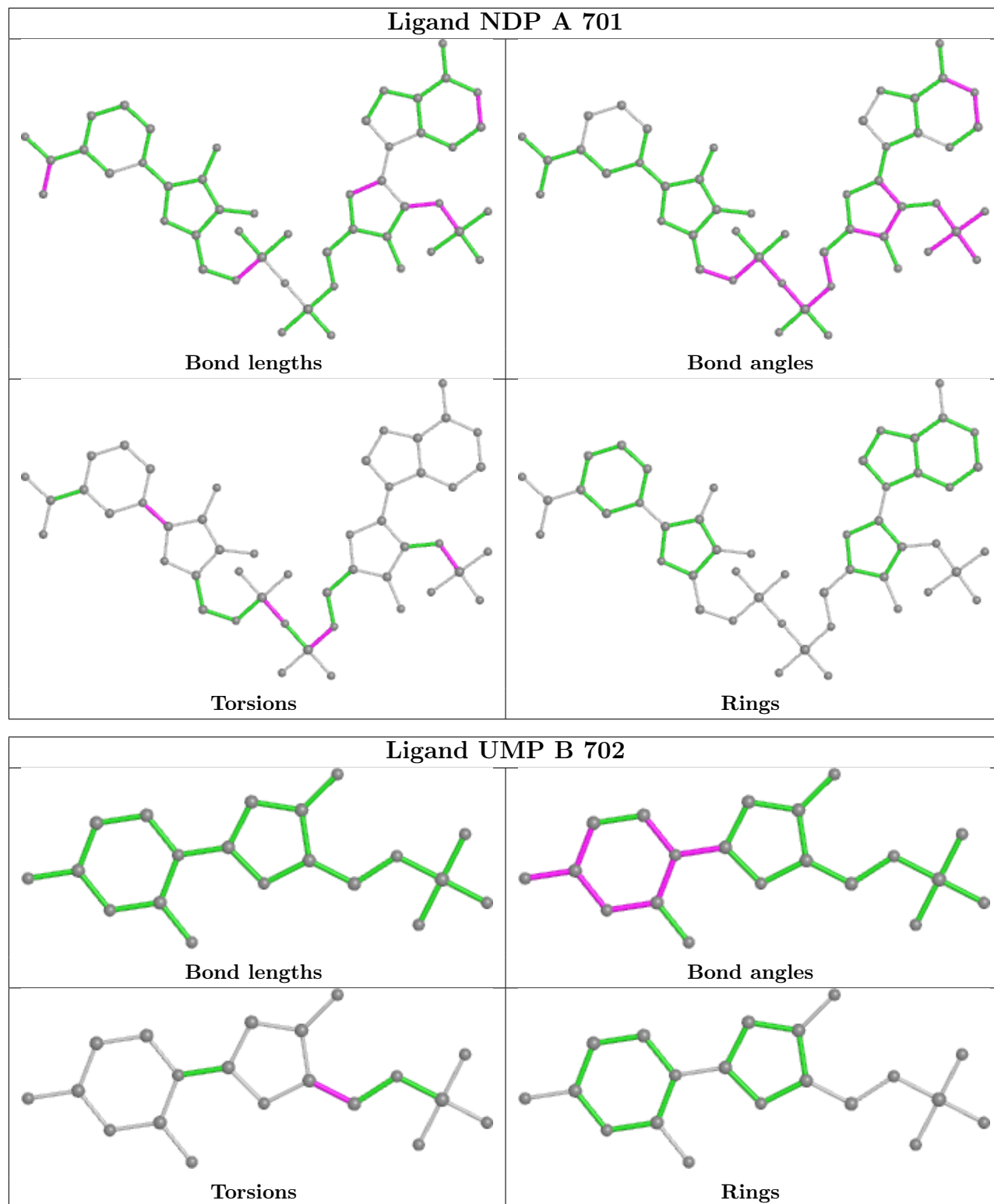
There are no ring outliers.

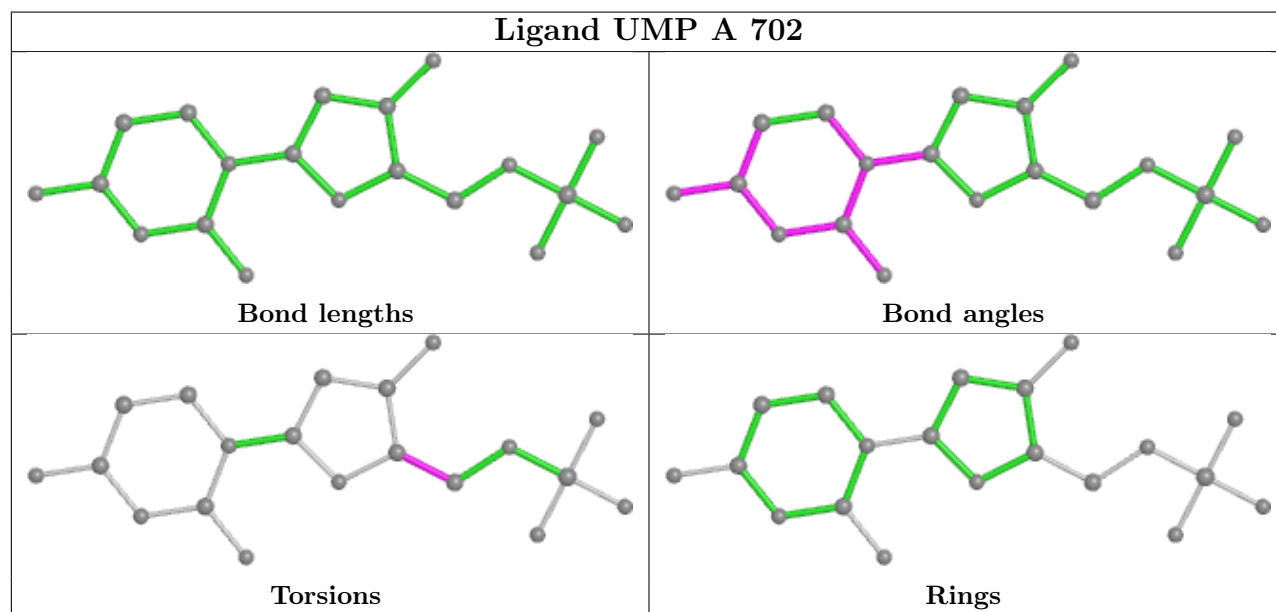
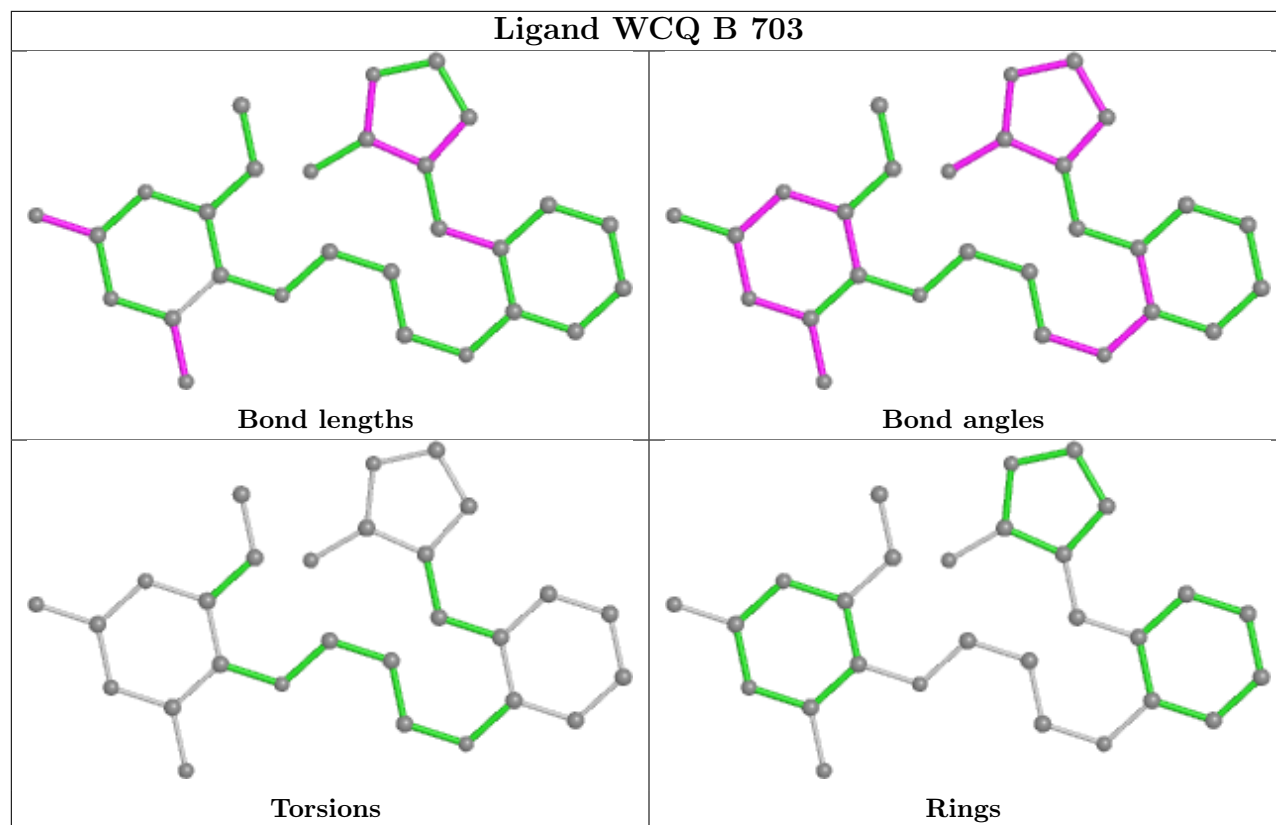
2 monomers are involved in 3 short contacts:

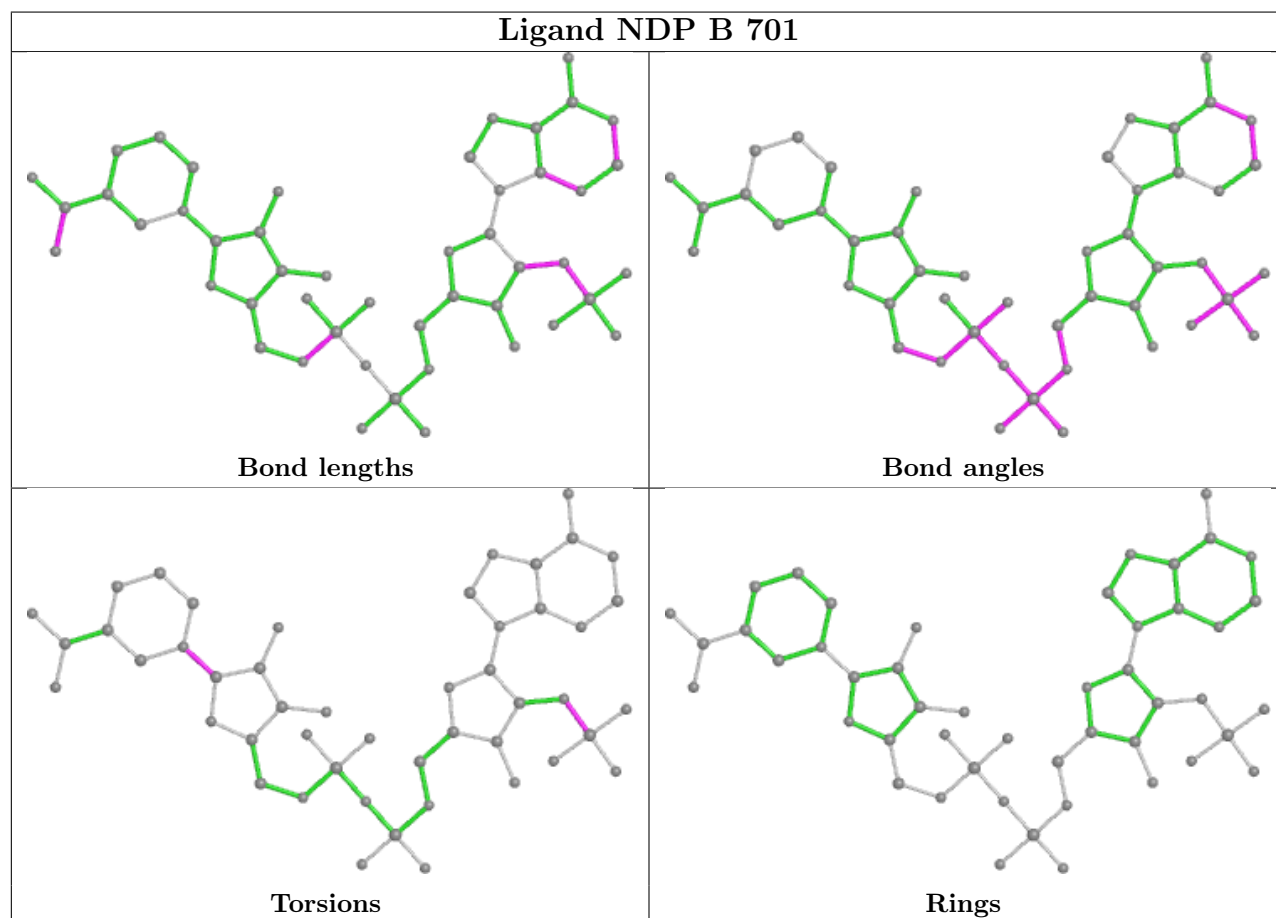
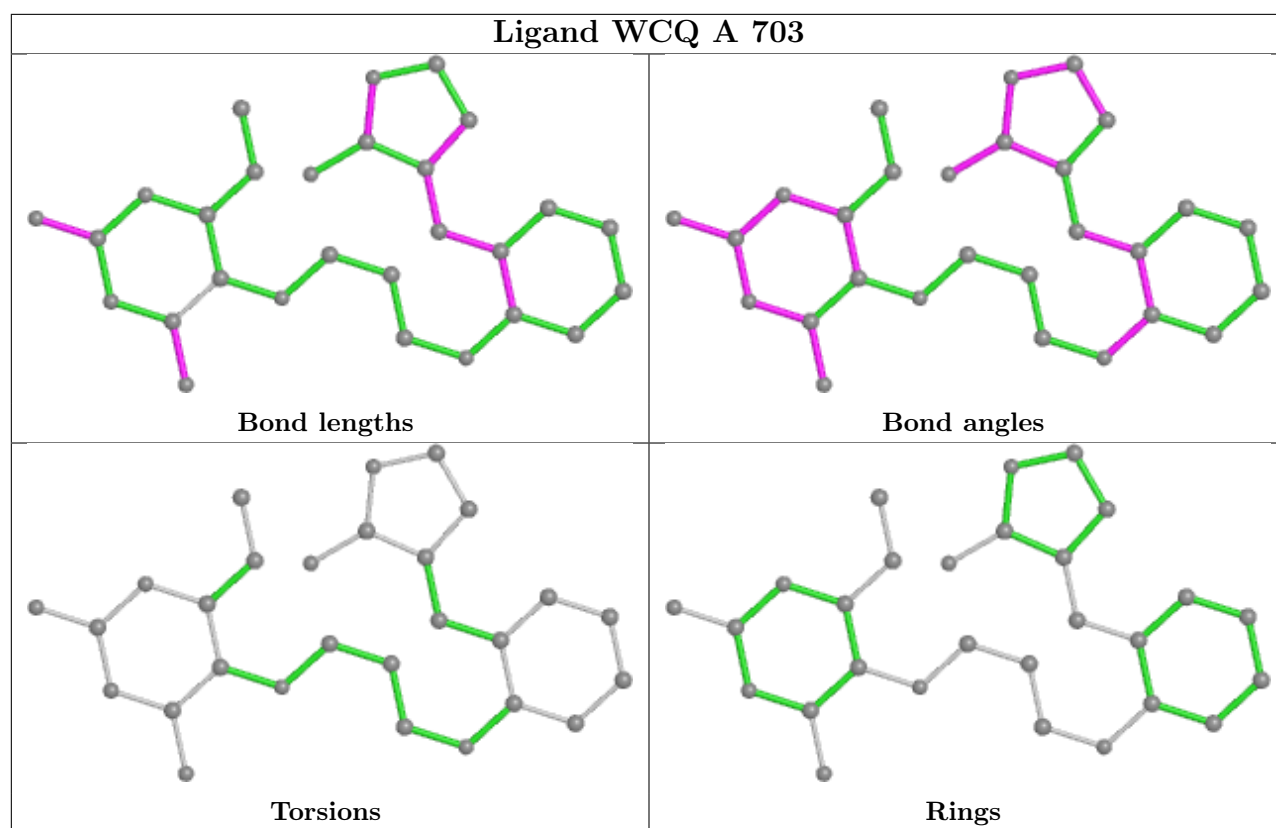
Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	701	NDP	1	0
2	B	701	NDP	2	0

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 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, 95<sup>th</sup> 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 <sup>2</sup> )	Q<0.9
1	A	532/608 (87%)	-0.06	27 (5%) 34 40	14, 27, 62, 87	0
1	B	528/608 (86%)	0.29	49 (9%) 16 19	14, 32, 70, 96	0
All	All	1060/1216 (87%)	0.12	76 (7%) 23 27	14, 29, 67, 96	0

The worst 5 of 76 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	B	307	ILE	4.3
1	B	308	HIS	4.2
1	A	85	THR	4.0
1	B	142	ILE	4.0
1	B	131	LEU	4.0

### 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](#)

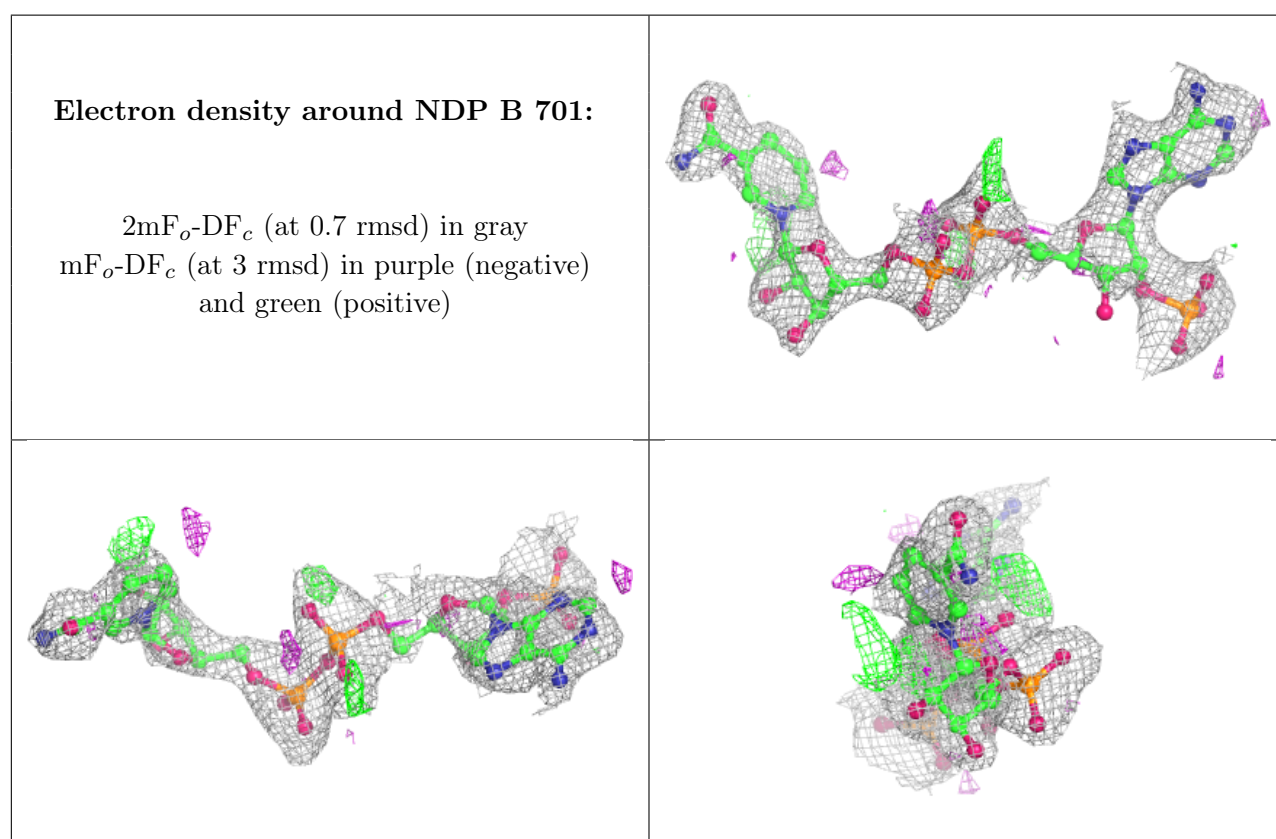
There are no monosaccharides in this entry.

### 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, 95<sup>th</sup> 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.

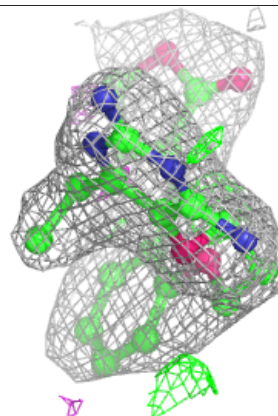
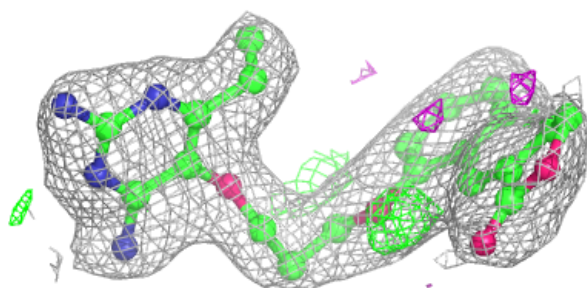
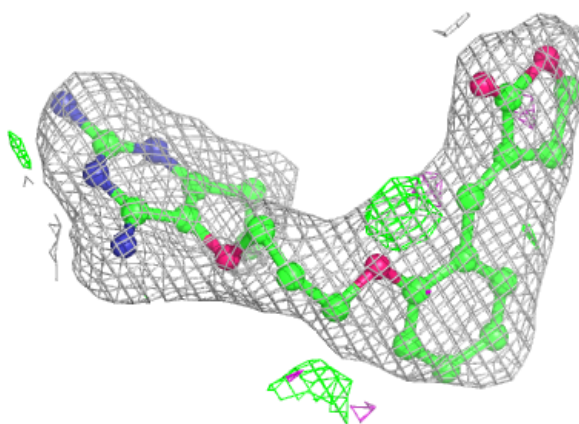
Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors( $\text{\AA}^2$ )	Q<0.9
2	NDP	B	701	48/48	0.82	0.14	39,63,76,78	0
4	WCQ	B	703	28/28	0.88	0.12	38,48,58,60	0
3	UMP	B	702	20/20	0.90	0.12	26,38,45,45	0
3	UMP	A	702	20/20	0.94	0.09	27,33,38,38	0
4	WCQ	A	703	28/28	0.95	0.07	17,24,35,36	0
2	NDP	A	701	48/48	0.96	0.08	22,32,39,41	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.

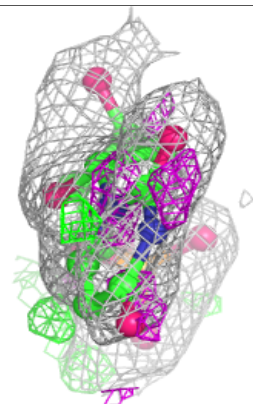
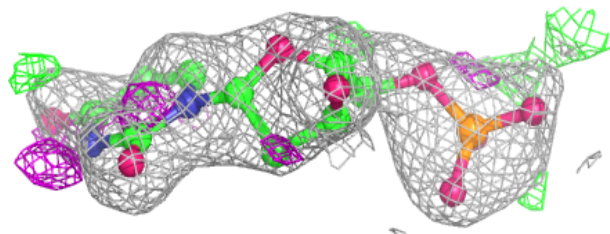
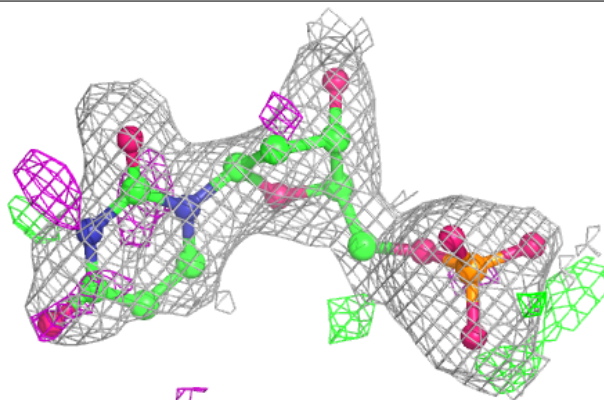


**Electron density around WCQ B 703:**

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

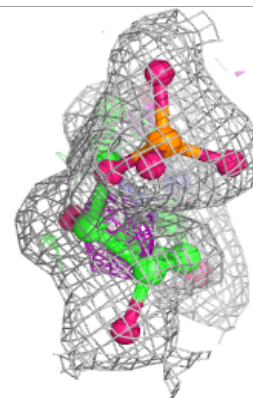
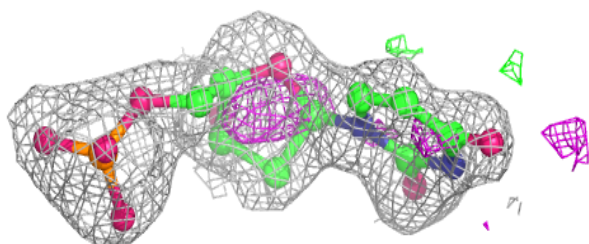
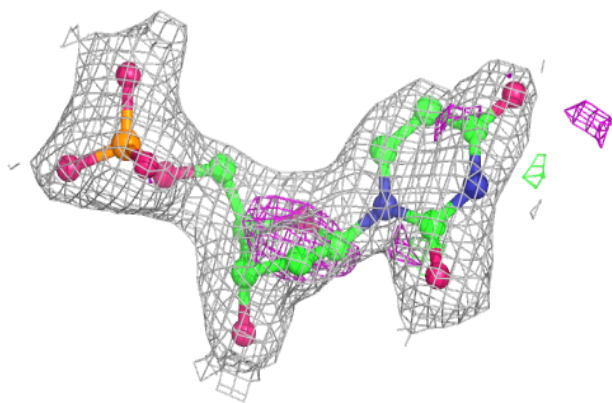
**Electron density around UMP B 702:**

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

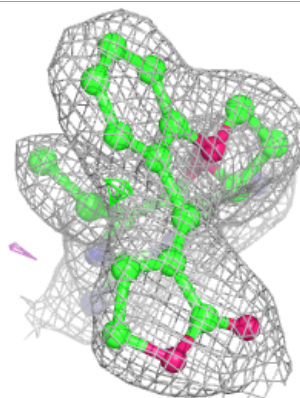
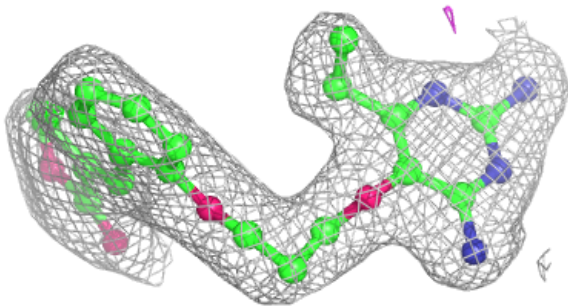
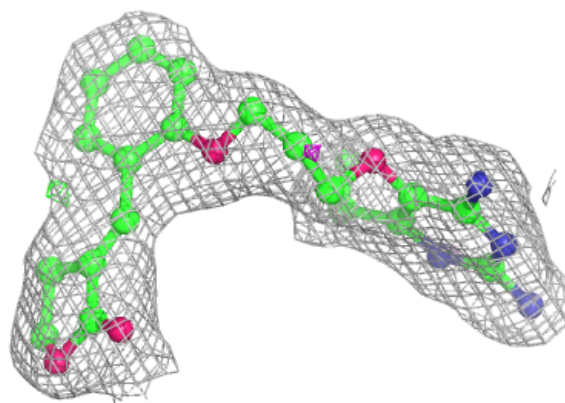


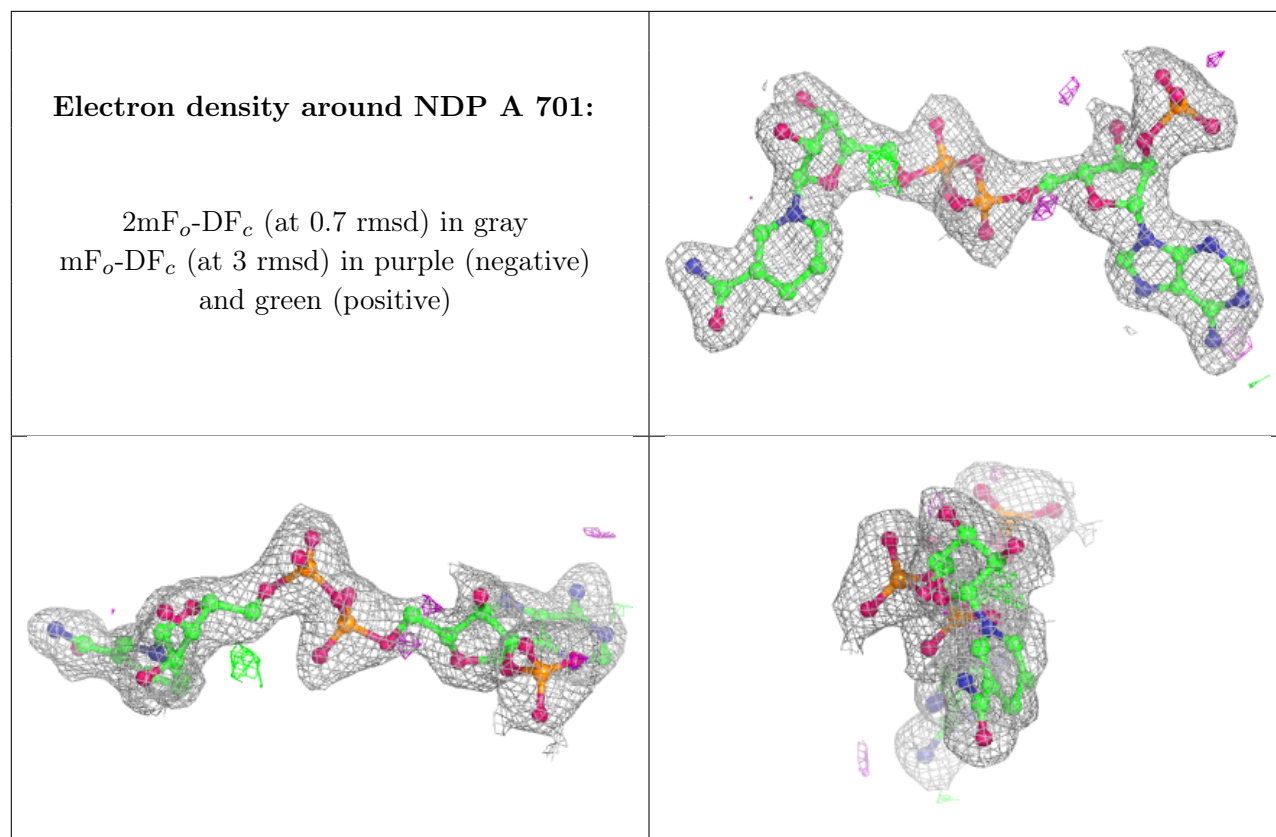
**Electron density around UMP A 702:**

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

**Electron density around WCQ A 703:**

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





## 6.5 Other polymers [i](#)

There are no such residues in this entry.