



# wwPDB X-ray Structure Validation Summary Report ⓘ

Oct 3, 2021 – 04:08 AM EDT

PDB ID : 3IX9  
Title : Crystal structure of Streptococcus pneumoniae dihydrofolate reductase - Sp9 mutant  
Authors : Yennawar, N.H.  
Deposited on : 2009-09-03  
Resolution : 1.95 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.

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

MolProbity : 4.02b-467  
Mogul : 1.8.5 (274361), CSD as541be (2020)  
Xtriage (Phenix) : 1.13  
EDS : 2.23.2  
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) : 2.23.2

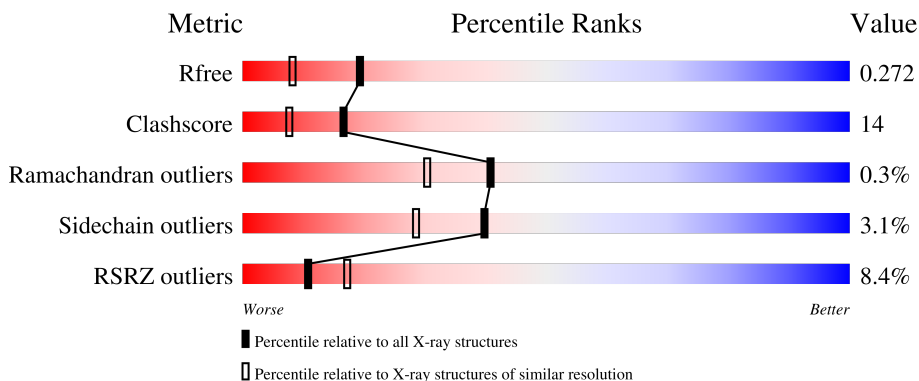
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*X-RAY DIFFRACTION*

The reported resolution of this entry is 1.95 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	2580 (1.96-1.96)
Clashscore	141614	2705 (1.96-1.96)
Ramachandran outliers	138981	2678 (1.96-1.96)
Sidechain outliers	138945	2678 (1.96-1.96)
RSRZ outliers	127900	2539 (1.96-1.96)

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	190	<div> <div>9%</div> <div> <div></div> <div>63%</div> <div>23%</div> <div>•</div> <div>13%</div> </div> </div>
1	B	190	<div> <div>6%</div> <div> <div></div> <div>66%</div> <div>19%</div> <div>•</div> <div>13%</div> </div> </div>

## 2 Entry composition

There are 4 unique types of molecules in this entry. The entry contains 3218 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 Dihydrofolate reductase.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	166	Total	C	N	O	S	0	0	0
			1375	888	231	254	2			
1	B	166	Total	C	N	O	S	0	0	0
			1375	888	231	254	2			

There are 62 discrepancies between the modelled and reference sequences:

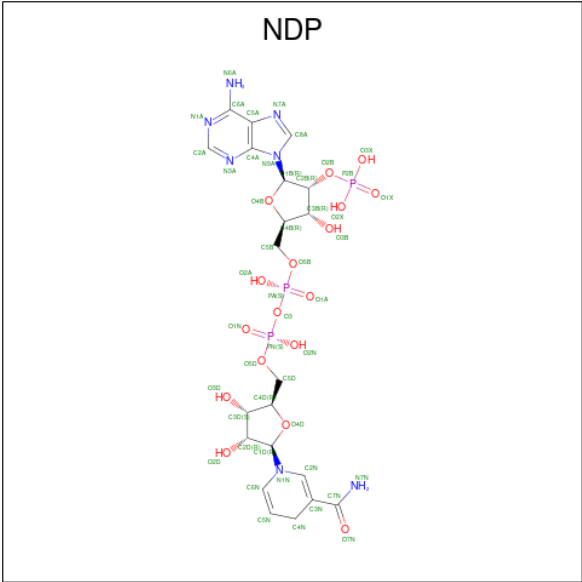
Chain	Residue	Modelled	Actual	Comment	Reference
A	-19	MET	-	expression tag	UNP O85261
A	-18	LYS	-	expression tag	UNP O85261
A	-17	HIS	-	expression tag	UNP O85261
A	-16	HIS	-	expression tag	UNP O85261
A	-15	HIS	-	expression tag	UNP O85261
A	-14	HIS	-	expression tag	UNP O85261
A	-13	HIS	-	expression tag	UNP O85261
A	-12	HIS	-	expression tag	UNP O85261
A	-11	HIS	-	expression tag	UNP O85261
A	-10	HIS	-	expression tag	UNP O85261
A	-9	GLY	-	expression tag	UNP O85261
A	-8	GLY	-	expression tag	UNP O85261
A	-7	LEU	-	expression tag	UNP O85261
A	-6	VAL	-	expression tag	UNP O85261
A	-5	PRO	-	expression tag	UNP O85261
A	-4	ARG	-	expression tag	UNP O85261
A	-3	GLY	-	expression tag	UNP O85261
A	-2	SER	-	expression tag	UNP O85261
A	-1	HIS	-	expression tag	UNP O85261
A	0	GLY	-	expression tag	UNP O85261
A	26	TYR	HIS	engineered mutation	UNP O85261
A	60	LYS	GLN	engineered mutation	UNP O85261
A	77	VAL	ALA	engineered mutation	UNP O85261
A	78	ALA	VAL	engineered mutation	UNP O85261
A	81	HIS	GLN	engineered mutation	UNP O85261

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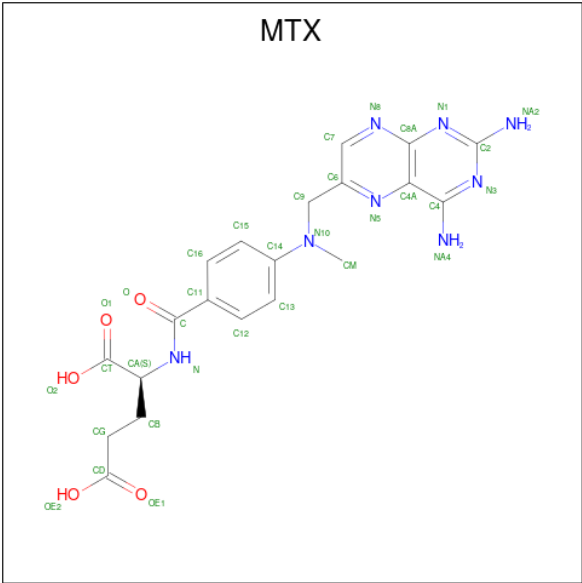
Chain	Residue	Modelled	Actual	Comment	Reference
A	91	SER	GLN	engineered mutation	UNP O85261
A	100	VAL	LEU	engineered mutation	UNP O85261
A	133	ALA	GLU	engineered mutation	UNP O85261
A	149	THR	ALA	engineered mutation	UNP O85261
A	169	LEU	-	expression tag	UNP O85261
A	170	GLU	-	expression tag	UNP O85261
B	-19	MET	-	expression tag	UNP O85261
B	-18	LYS	-	expression tag	UNP O85261
B	-17	HIS	-	expression tag	UNP O85261
B	-16	HIS	-	expression tag	UNP O85261
B	-15	HIS	-	expression tag	UNP O85261
B	-14	HIS	-	expression tag	UNP O85261
B	-13	HIS	-	expression tag	UNP O85261
B	-12	HIS	-	expression tag	UNP O85261
B	-11	HIS	-	expression tag	UNP O85261
B	-10	HIS	-	expression tag	UNP O85261
B	-9	GLY	-	expression tag	UNP O85261
B	-8	GLY	-	expression tag	UNP O85261
B	-7	LEU	-	expression tag	UNP O85261
B	-6	VAL	-	expression tag	UNP O85261
B	-5	PRO	-	expression tag	UNP O85261
B	-4	ARG	-	expression tag	UNP O85261
B	-3	GLY	-	expression tag	UNP O85261
B	-2	SER	-	expression tag	UNP O85261
B	-1	HIS	-	expression tag	UNP O85261
B	0	GLY	-	expression tag	UNP O85261
B	26	TYR	HIS	engineered mutation	UNP O85261
B	60	LYS	GLN	engineered mutation	UNP O85261
B	77	VAL	ALA	engineered mutation	UNP O85261
B	78	ALA	VAL	engineered mutation	UNP O85261
B	81	HIS	GLN	engineered mutation	UNP O85261
B	91	SER	GLN	engineered mutation	UNP O85261
B	100	VAL	LEU	engineered mutation	UNP O85261
B	133	ALA	GLU	engineered mutation	UNP O85261
B	149	THR	ALA	engineered mutation	UNP O85261
B	169	LEU	-	expression tag	UNP O85261
B	170	GLU	-	expression tag	UNP O85261

- 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
2	A	1	Total 48	C 21	N 7	O 17	P 3	0	0
2	B	1	Total 48	C 21	N 7	O 17	P 3	0	0

- Molecule 3 is METHOTREXATE (three-letter code: MTX) (formula: C<sub>20</sub>H<sub>22</sub>N<sub>8</sub>O<sub>5</sub>).



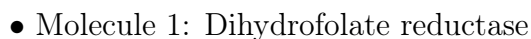
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
3	A	1	Total	C	N	O	0	0
			33	20	8	5		
3	B	1	Total	C	N	O	0	0
			33	20	8	5		

- Molecule 4 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
4	A	164	Total 164	O 164	0	0
4	B	142	Total 142	O 142	0	0



- Molecule 1: Dihydrofolate reductase



## 4 Data and refinement statistics

Property	Value	Source
Space group	P 21 21 2	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	61.84Å 93.64Å 71.37Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	20.00 – 1.95 39.15 – 1.95	Depositor EDS
% Data completeness (in resolution range)	92.6 (20.00-1.95) 92.6 (39.15-1.95)	Depositor EDS
$R_{merge}$	0.10	Depositor
$R_{sym}$	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	2.01 (at 1.95Å)	Xtriage
Refinement program	CNS 1.2	Depositor
R, $R_{free}$	0.237 , 0.274 0.230 , 0.272	Depositor DCC
$R_{free}$ test set	1427 reflections (4.98%)	wwPDB-VP
Wilson B-factor (Å <sup>2</sup> )	29.2	Xtriage
Anisotropy	0.335	Xtriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.36 , 53.3	EDS
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.52$ , $\langle L^2 \rangle = 0.36$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
$F_o, F_c$ correlation	0.94	EDS
Total number of atoms	3218	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	31.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 9.02% 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, MTX

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	0.54	0/1410	0.72	1/1908 (0.1%)
1	B	0.50	0/1410	0.71	2/1908 (0.1%)
All	All	0.52	0/2820	0.72	3/3816 (0.1%)

There are no bond length outliers.

All (3) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	B	102	GLY	N-CA-C	-6.12	97.79	113.10
1	A	100	VAL	C-N-CA	-5.81	110.10	122.30
1	B	100	VAL	C-N-CA	-5.59	110.55	122.30

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	1375	0	1349	39	0
1	B	1375	0	1349	40	0
2	A	48	0	26	7	0
2	B	48	0	26	6	0
3	A	33	0	20	1	0
3	B	33	0	20	4	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
4	A	164	0	0	2	0
4	B	142	0	0	4	0
All	All	3218	0	2790	81	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 14.

The worst 5 of 81 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:101:GLY:HA3	2:B:193:NDP:H5N	1.66	0.78
1:B:143:VAL:HG11	1:B:164:LYS:HG3	1.72	0.72
1:B:69:ASN:OD1	1:B:71:GLU:HG2	1.90	0.71
1:A:93:GLN:HE22	1:A:95:LYS:HB2	1.57	0.70
1:A:101:GLY:HA3	2:A:193:NDP:H5N	1.74	0.69

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	164/190 (86%)	158 (96%)	5 (3%)	1 (1%)	25	14
1	B	164/190 (86%)	162 (99%)	2 (1%)	0	100	100
All	All	328/380 (86%)	320 (98%)	7 (2%)	1 (0%)	41	30

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	74	ILE

### 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	147/168 (88%)	143 (97%)	4 (3%)	44	34
1	B	147/168 (88%)	142 (97%)	5 (3%)	37	25
All	All	294/336 (88%)	285 (97%)	9 (3%)	40	28

5 of 9 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	61	ARG
1	B	71	GLU
1	A	138	SER
1	B	12	ASP
1	B	22	ARG

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 7 such sidechains are listed below:

Mol	Chain	Res	Type
1	A	93	GLN
1	A	122	HIS
1	B	41	HIS
1	B	40	ASN
1	A	81	HIS

### 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 [i](#)

There are no monosaccharides in this entry.

## 5.6 Ligand geometry [i](#)

4 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	MTX	A	200	-	29,35,35	2.46	3 (10%)	38,49,49	1.94	9 (23%)
2	NDP	B	193	-	45,52,52	1.37	5 (11%)	53,80,80	1.14	3 (5%)
3	MTX	B	200	-	29,35,35	2.47	3 (10%)	38,49,49	1.99	10 (26%)
2	NDP	A	193	-	45,52,52	1.39	5 (11%)	53,80,80	1.16	4 (7%)

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	MTX	A	200	-	-	0/19/25/25	0/3/3/3
2	NDP	B	193	-	-	3/30/77/77	0/5/5/5
3	MTX	B	200	-	-	1/19/25/25	0/3/3/3
2	NDP	A	193	-	-	2/30/77/77	0/5/5/5

The worst 5 of 16 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	B	200	MTX	CM-N10	-7.85	1.32	1.46
3	A	200	MTX	CM-N10	-7.83	1.32	1.46
3	B	200	MTX	C9-N10	-7.05	1.32	1.46
3	A	200	MTX	C9-N10	-6.98	1.32	1.46
3	A	200	MTX	C9-C6	-6.94	1.39	1.51

The worst 5 of 26 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
3	B	200	MTX	N8-C8A-N1	5.18	121.74	115.82
3	A	200	MTX	N8-C8A-N1	4.82	121.32	115.82
2	B	193	NDP	N3A-C2A-N1A	-4.74	121.26	128.68
3	A	200	MTX	C2-N1-C8A	4.68	120.70	115.36
3	A	200	MTX	N1-C2-N3	-4.66	121.00	127.22

There are no chirality outliers.

5 of 6 torsion outliers are listed below:

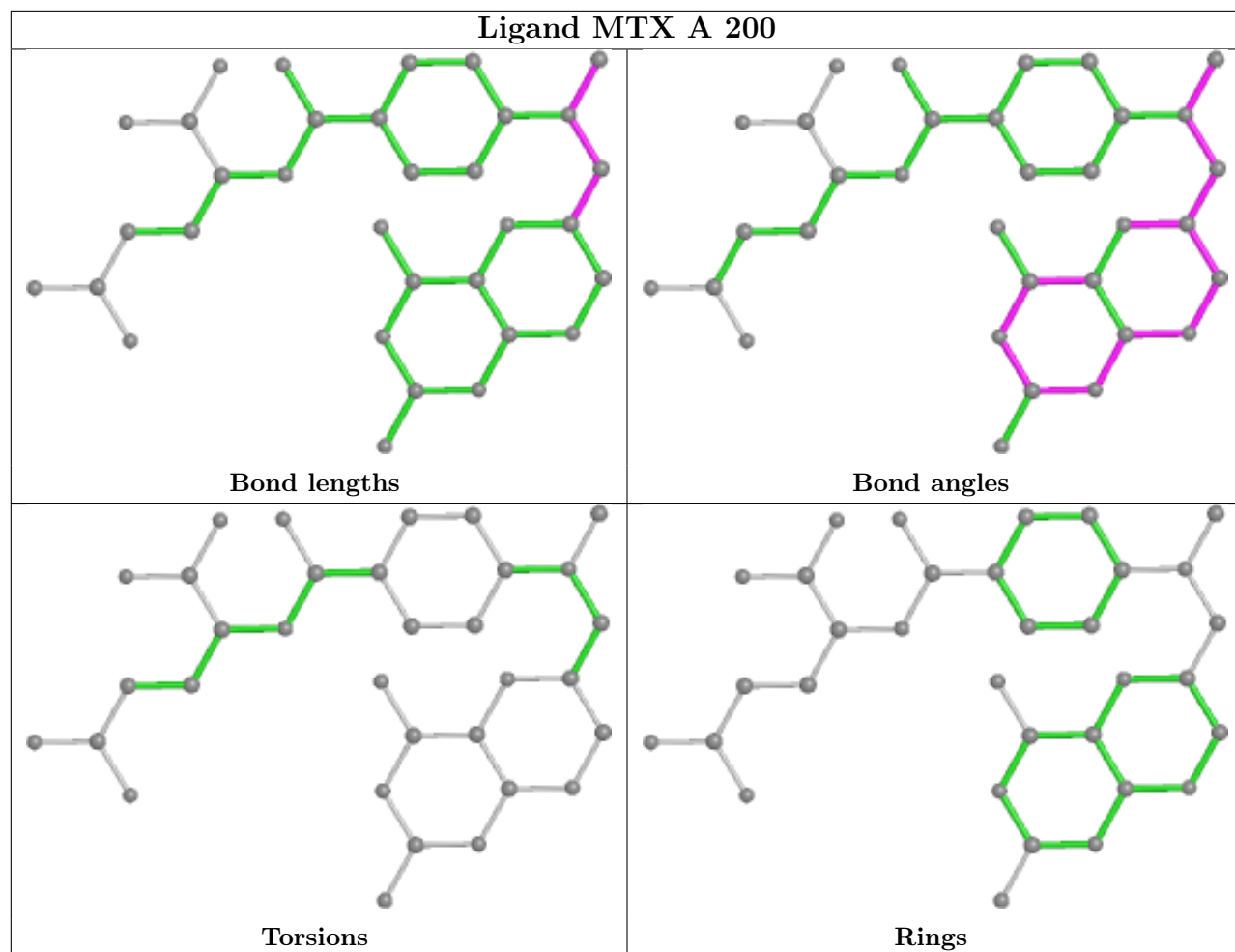
Mol	Chain	Res	Type	Atoms
3	B	200	MTX	C6-C9-N10-CM
2	A	193	NDP	O4D-C1D-N1N-C2N
2	B	193	NDP	O4D-C1D-N1N-C2N
2	B	193	NDP	C2D-C1D-N1N-C2N
2	A	193	NDP	C2D-C1D-N1N-C2N

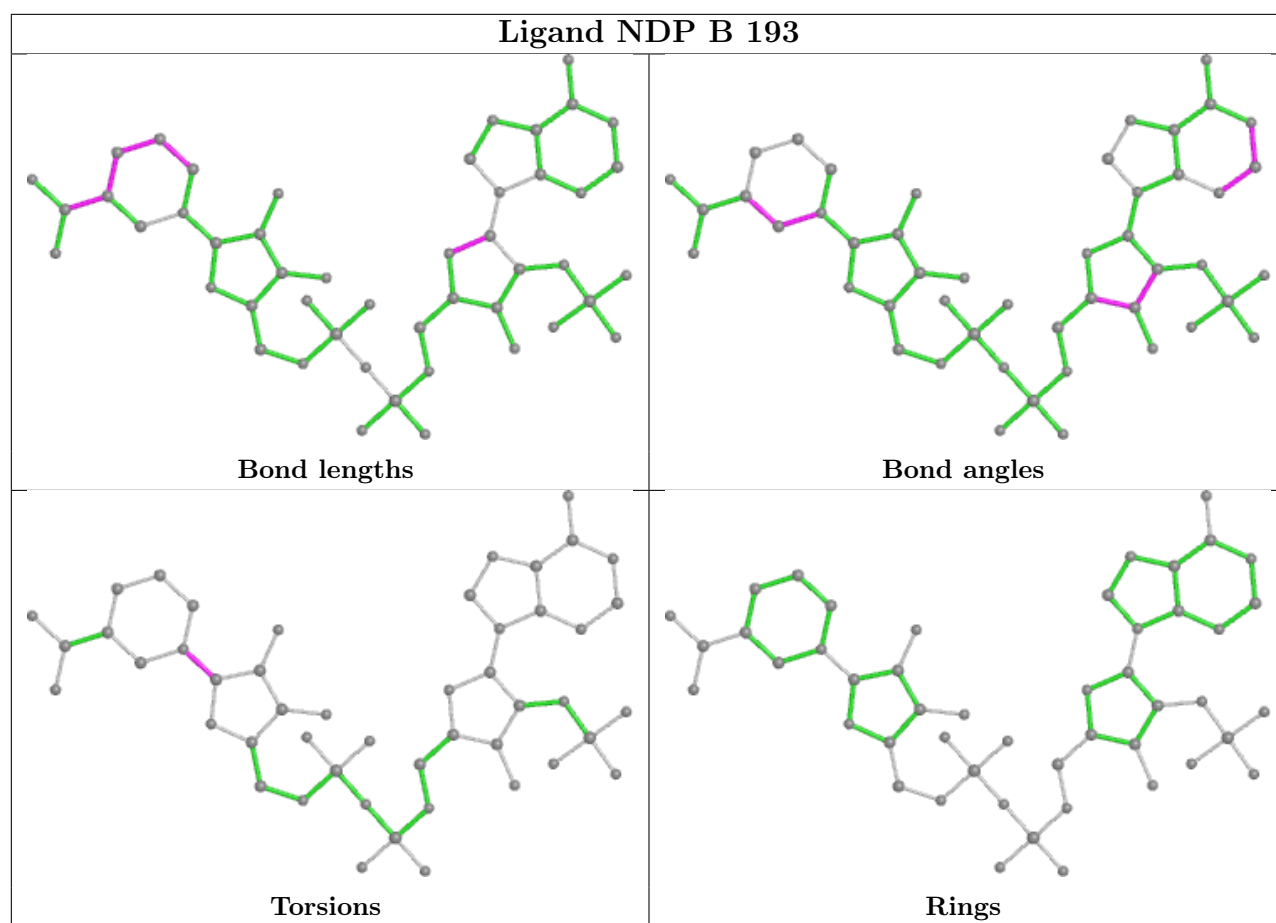
There are no ring outliers.

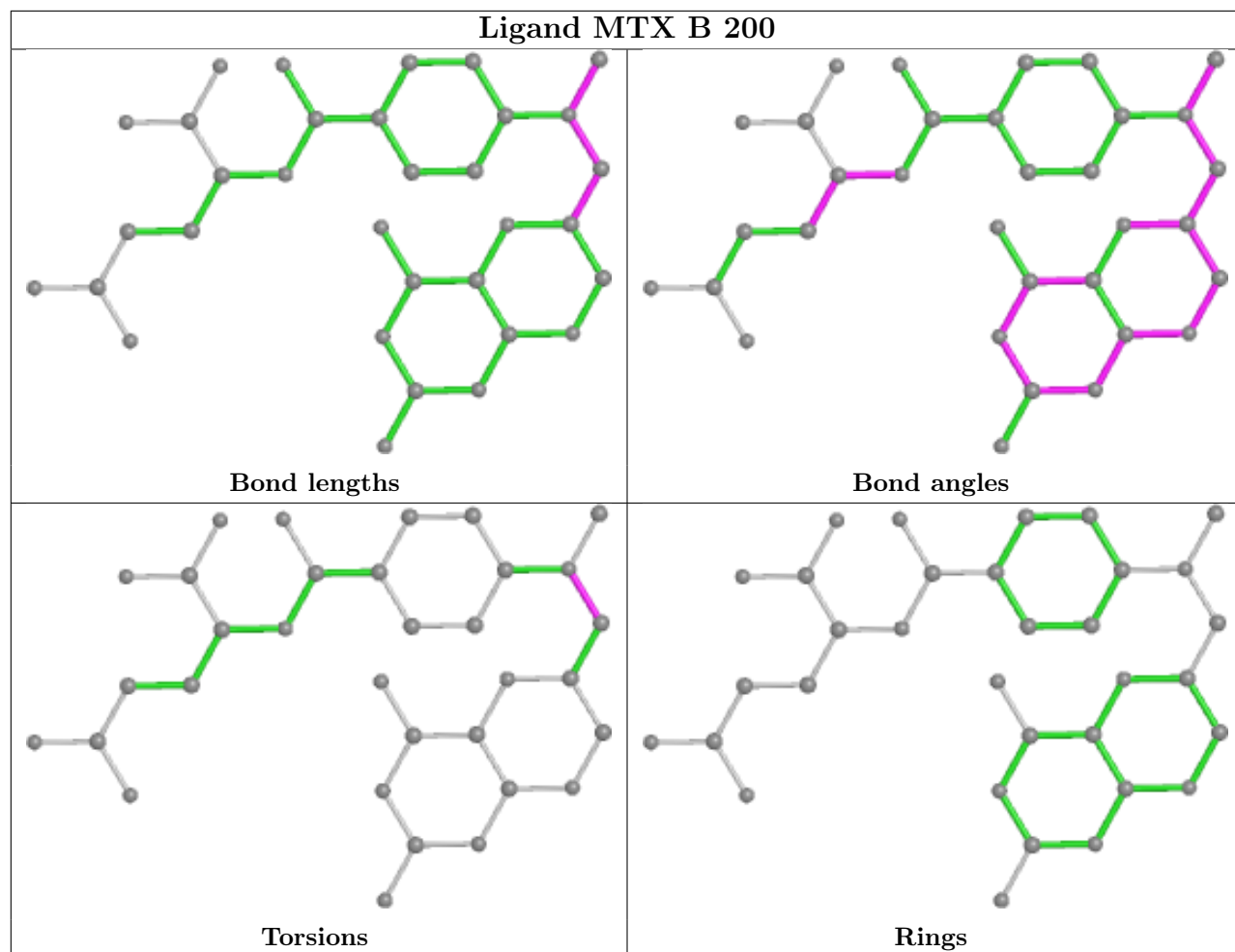
4 monomers are involved in 18 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	A	200	MTX	1	0
2	B	193	NDP	6	0
3	B	200	MTX	4	0
2	A	193	NDP	7	0

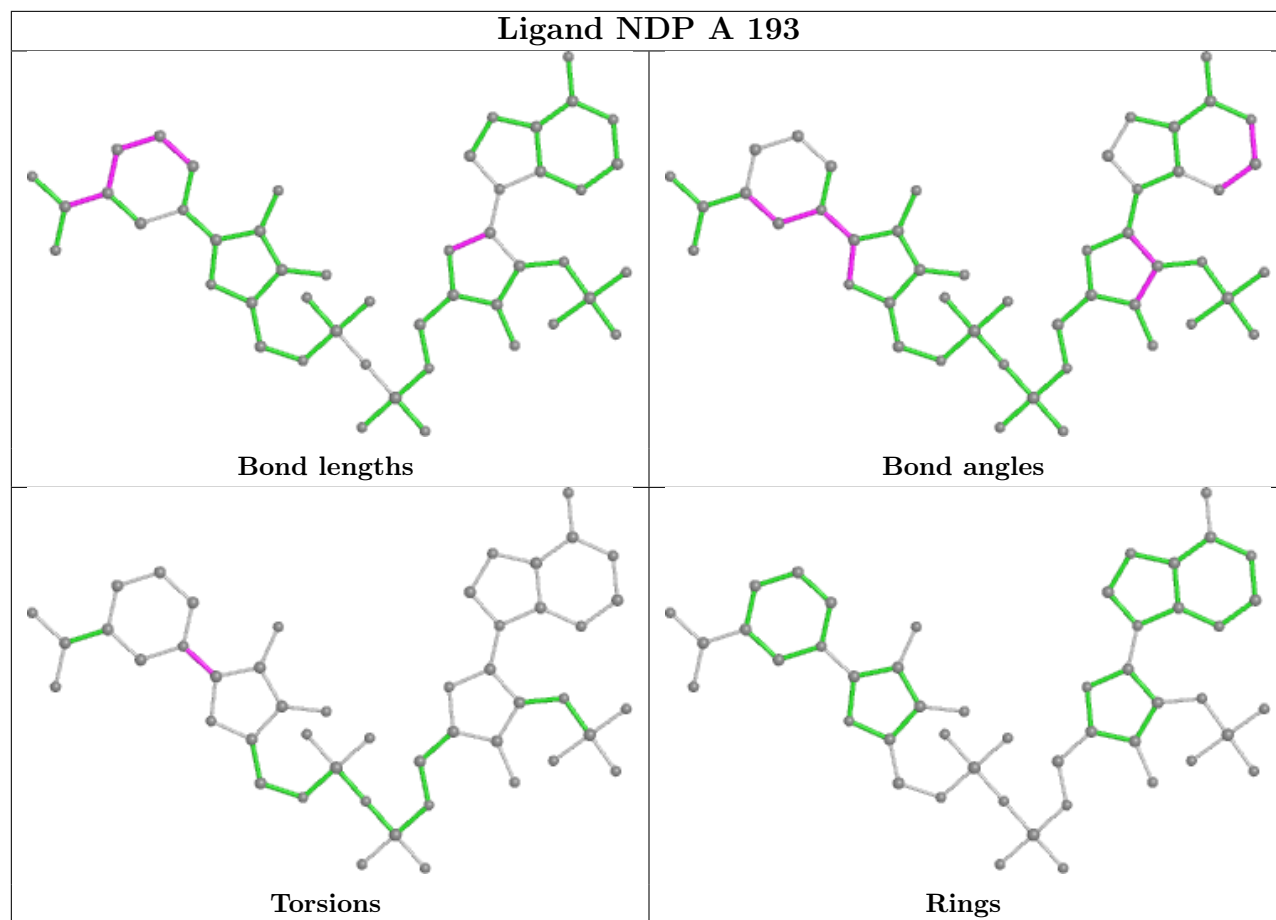
The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. 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	166/190 (87%)	0.73	17 (10%) 6 11	19, 28, 48, 58	0
1	B	166/190 (87%)	0.72	11 (6%) 18 26	20, 29, 45, 56	0
All	All	332/380 (87%)	0.72	28 (8%) 11 17	19, 29, 47, 58	0

The worst 5 of 28 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	135	PHE	5.9
1	B	2	THR	5.9
1	B	167	GLU	5.8
1	A	74	ILE	5.3
1	A	139	LEU	5.3

### 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](#)

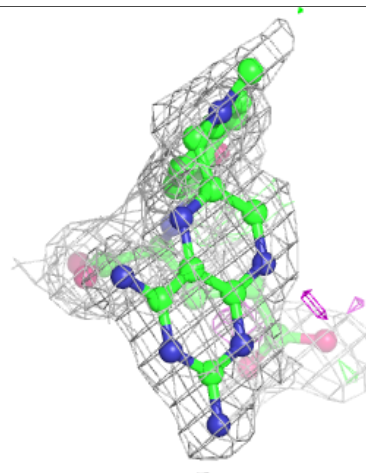
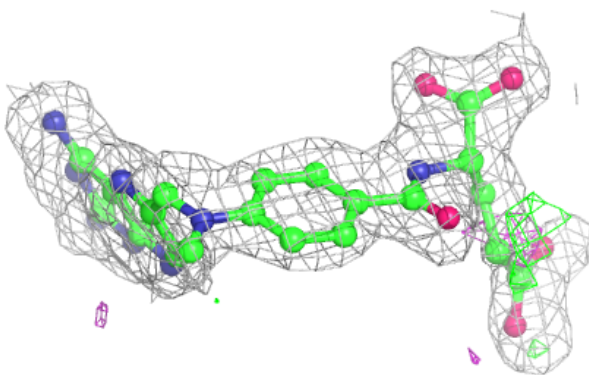
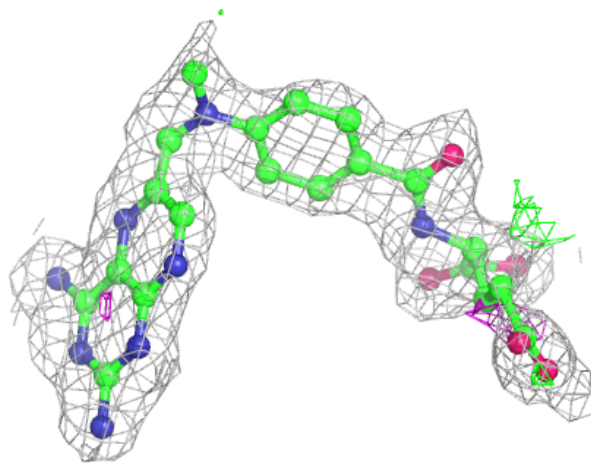
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95<sup>th</sup> percentile and maximum values of B factors of atoms in the group. The column labelled ‘Q< 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
3	MTX	B	200	33/33	0.90	0.16	20,28,45,48	0
3	MTX	A	200	33/33	0.93	0.12	19,24,29,29	0
2	NDP	A	193	48/48	0.95	0.11	19,26,30,31	0
2	NDP	B	193	48/48	0.95	0.12	18,25,30,30	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.

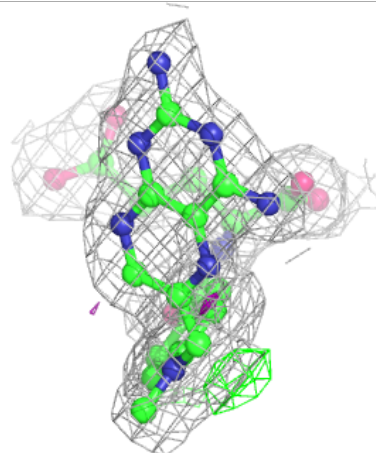
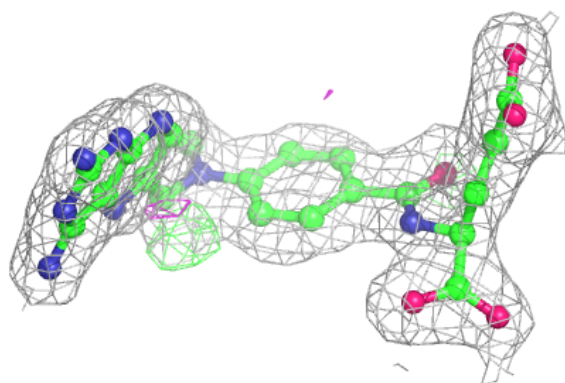
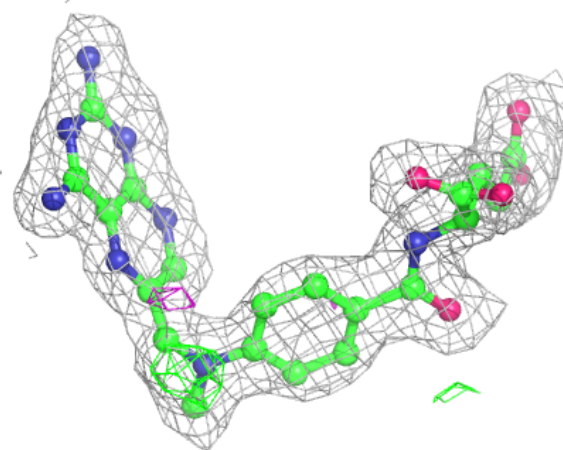
**Electron density around MTX B 200:**

2mF<sub>o</sub>-DF<sub>c</sub> (at 0.7 rmsd) in gray  
mF<sub>o</sub>-DF<sub>c</sub> (at 3 rmsd) in purple (negative)  
and green (positive)



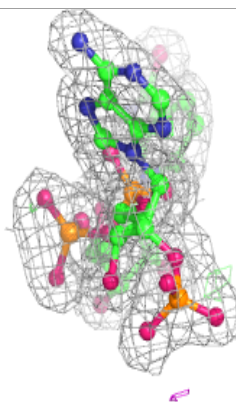
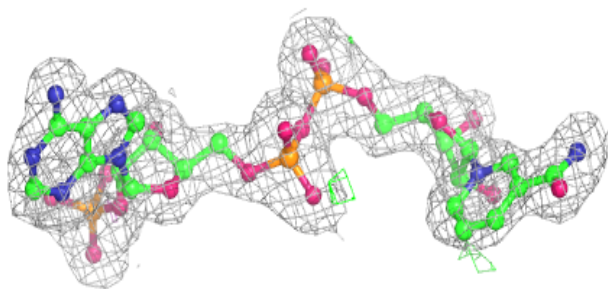
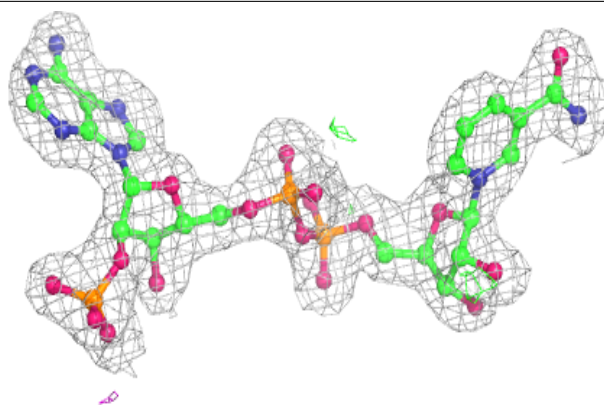
**Electron density around MTX A 200:**

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

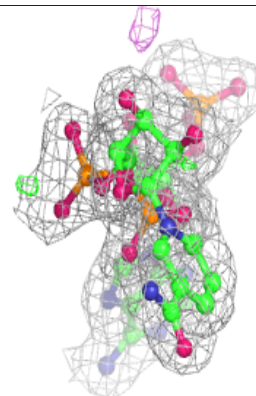
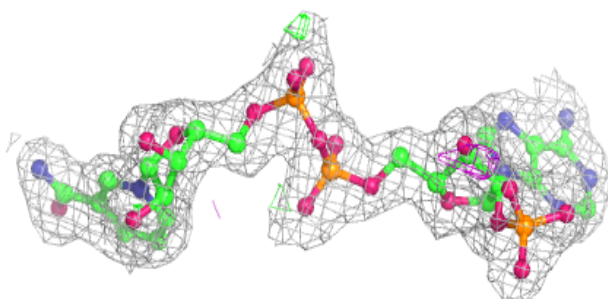
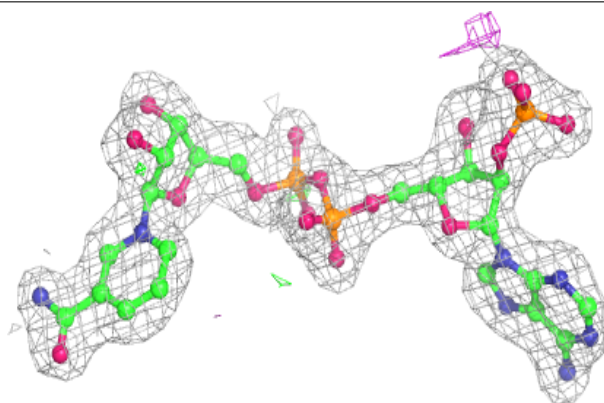


**Electron density around NDP A 193:**

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

**Electron density around NDP B 193:**

$2mF_o-DF_c$  (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.