



Full wwPDB X-ray Structure Validation Report ⓘ

Feb 19, 2025 – 11:57 am GMT

PDB ID : 9IFT
Title : FSP1 (tetrapod ancestor) bound to FAD and NAD⁺
Deposited on : 2025-02-18
Resolution : 1.45 Å (reported)

This wwPDB validation report is for manuscript review

This is a Full wwPDB X-ray Structure Validation Report.

This report is produced by the wwPDB biocuration pipeline after annotation of the structure.

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 ⓘ symbol.

The types of validation reports are described at

<https://www.wwpdb.org/validation/2017/FAQs#types>.

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

MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (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	:	9.0.003 (Gargrove)
Density-Fitness	:	1.0.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)

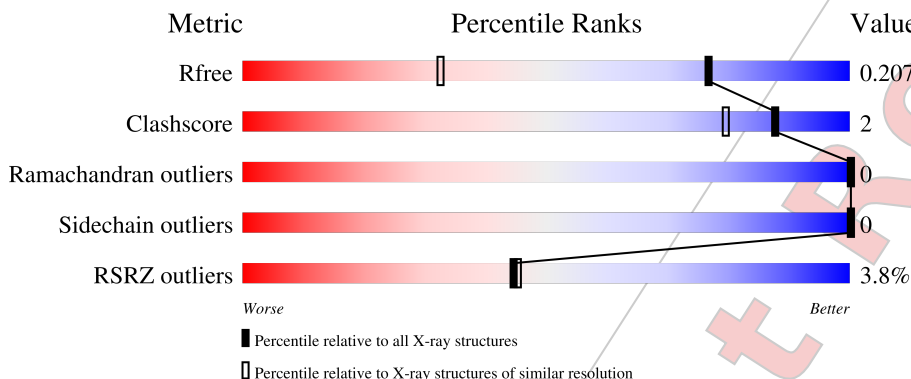
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

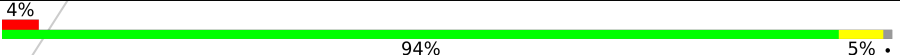
The reported resolution of this entry is 1.45 Å.

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	1556 (1.46-1.46)
Clashscore	180529	1653 (1.46-1.46)
Ramachandran outliers	177936	1635 (1.46-1.46)
Sidechain outliers	177891	1635 (1.46-1.46)
RSRZ outliers	164620	1556 (1.46-1.46)

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 $\leq 5\%$. 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	368	

2 Entry composition [i](#)

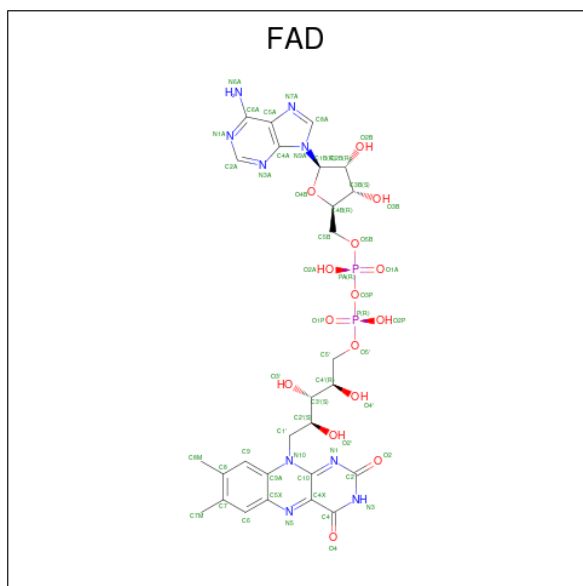
There are 6 unique types of molecules in this entry. The entry contains 3208 atoms, of which 0 are hydrogens and 0 are deuteriums.

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 FSP1 (tetrapod ancestor).

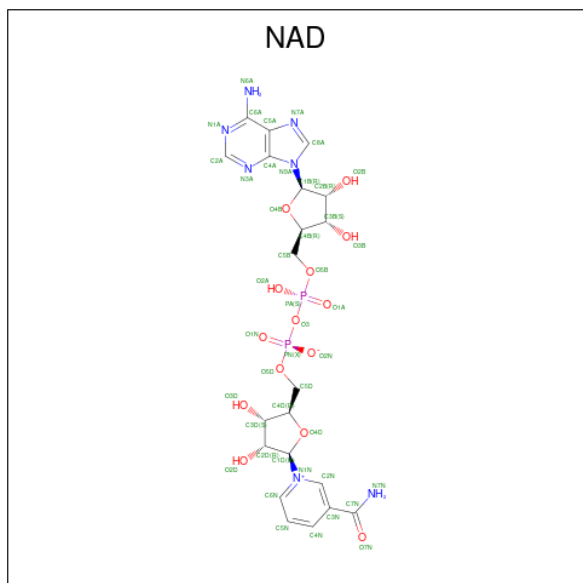
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	364	2803	1780	478	529	16	0	6	0

- Molecule 2 is FLAVIN-ADENINE DINUCLEOTIDE (three-letter code: FAD) (formula: $C_{27}H_{33}N_9O_{15}P_2$) (labeled as "Ligand of Interest" by depositor).



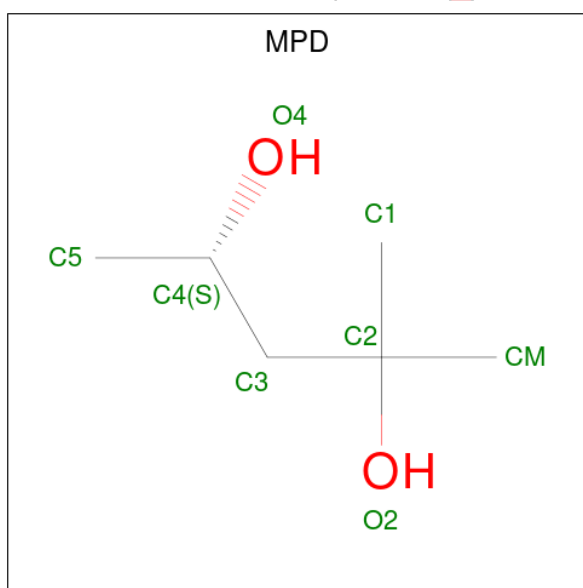
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
			Total	C	N	O	P		
2	A	1	53	27	9	15	2	0	0

- Molecule 3 is NICOTINAMIDE-ADENINE-DINUCLEOTIDE (three-letter code: NAD) (formula: $C_{21}H_{27}N_7O_{14}P_2$) (labeled as "Ligand of Interest" by depositor).



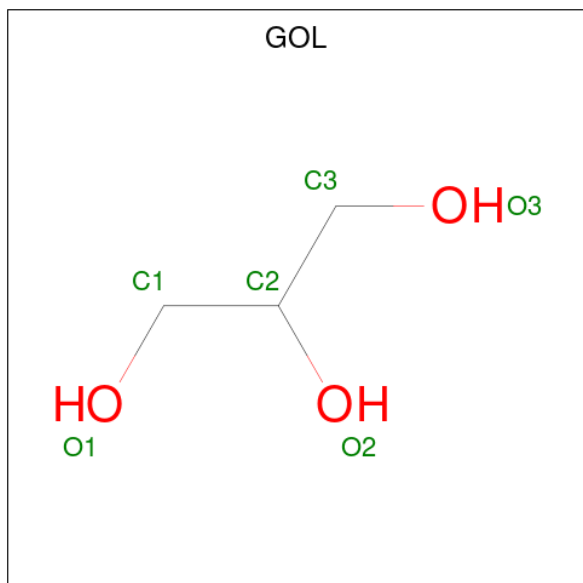
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
3	A	1	Total	C	N	O	P	
			44	21	7	14	2	

- Molecule 4 is (4S)-2-METHYL-2,4-PENTANEDIOL (three-letter code: MPD) (formula: $C_6H_{14}O_2$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
4	A	1	Total	C	O		
			8	6	2		

- Molecule 5 is GLYCEROL (three-letter code: GOL) (formula: $C_3H_8O_3$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
5	A	1	Total	C	O	0	0
			6	3	3		

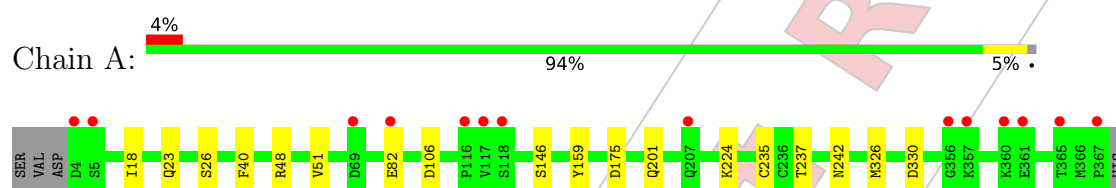
- Molecule 6 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
6	A	294	Total	O	0	0
			294	294		

3 Residue-property plots [i](#)

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: FSP1 (tetrapod ancestor)



4 Data and refinement statistics

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α , β , γ	43.95Å 80.53Å 60.92Å 90.00° 100.69° 90.00°	Depositor
Resolution (Å)	48.09 – 1.45 48.09 – 1.45	Depositor EDS
% Data completeness (in resolution range)	98.9 (48.09-1.45) 98.9 (48.09-1.45)	Depositor EDS
R_{merge}	0.10	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.48 (at 1.45Å)	Xtriage
Refinement program	REFMAC 5.8.0405	Depositor
R, R_{free}	0.176 , 0.203 0.181 , 0.207	Depositor DCC
R_{free} test set	2298 reflections (3.12%)	wwPDB-VP
Wilson B-factor (Å ²)	17.3	Xtriage
Anisotropy	0.076	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.35 , 34.5	EDS
L-test for twinning ²	$\langle L \rangle = 0.50$, $\langle L^2 \rangle = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.97	EDS
Total number of atoms	3208	wwPDB-VP
Average B, all atoms (Å ²)	22.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 6.61% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical 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: NAD, GOL, FAD, MPD

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	0.51	0/2850	0.82	2/3847 (0.1%)

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	1

There are no bond length outliers.

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	326	MET	CG-SD-CE	-12.09	80.86	100.20
1	A	330	ASP	CB-CG-OD2	-5.25	113.58	118.30

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	48	ARG	Sidechain

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	2803	0	2840	11	0
2	A	53	0	31	0	0
3	A	44	0	26	0	0
4	A	8	0	14	0	0
5	A	6	0	8	0	0
6	A	294	0	0	1	1
All	All	3208	0	2919	11	1

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 2.

All (11) 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:235[B]:CYS:HG	1:A:237:THR:HG1	1.34	0.71
1:A:106:ASP:HB2	1:A:242:ASN:HD21	1.57	0.70
1:A:235[B]:CYS:SG	1:A:237:THR:OG1	2.46	0.69
1:A:82:GLU:OE1	6:A:502:HOH:O	2.15	0.62
1:A:146[A]:SER:OG	1:A:175:ASP:OD2	2.19	0.57
1:A:18[B]:ILE:HD12	1:A:40:PHE:CD1	2.47	0.49
1:A:23:GLN:O	1:A:26:SER:HB3	2.16	0.46
1:A:106:ASP:HB2	1:A:242:ASN:ND2	2.28	0.45
1:A:18[B]:ILE:HD11	1:A:40:PHE:HB2	1.99	0.45
1:A:201:GLN:HB3	1:A:224:LYS:HG3	2.00	0.44
1:A:51:VAL:HG13	1:A:159:TYR:HE2	1.83	0.44

All (1) symmetry-related close contacts are listed below. The label for Atom-2 includes the symmetry operator and encoded unit-cell translations to be applied.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
6:A:768:HOH:O	6:A:772:HOH:O[2_547]	1.60	0.60

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	368/368 (100%)	358 (97%)	10 (3%)	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	A	303/302 (100%)	303 (100%)	0	100	100

There are no protein residues with a non-rotameric sidechain to report.

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	242	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 oligosaccharides in this entry.

5.6 Ligand geometry ⓘ

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
5	GOL	A	404	-	5,5,5	0.27	0	5,5,5	0.79	0
4	MPD	A	403	-	7,7,7	0.27	0	9,10,10	0.44	0
3	NAD	A	402	-	42,48,48	0.72	0	50,73,73	0.89	1 (2%)
2	FAD	A	401	-	53,58,58	0.95	3 (5%)	68,89,89	0.87	1 (1%)

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	GOL	A	404	-	-	2/4/4/4	-
4	MPD	A	403	-	-	4/5/5/5	-
3	NAD	A	402	-	-	1/26/62/62	0/5/5/5
2	FAD	A	401	-	-	4/30/50/50	0/6/6/6

All (3) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	401	FAD	C6-C7	-2.53	1.35	1.39
2	A	401	FAD	C1'-C2'	-2.32	1.49	1.52
2	A	401	FAD	C4X-C4	-2.01	1.37	1.44

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
3	A	402	NAD	O4B-C1B-C2B	-2.75	102.90	106.93
2	A	401	FAD	O2A-PA-O1A	2.13	122.79	112.24

There are no chirality outliers.

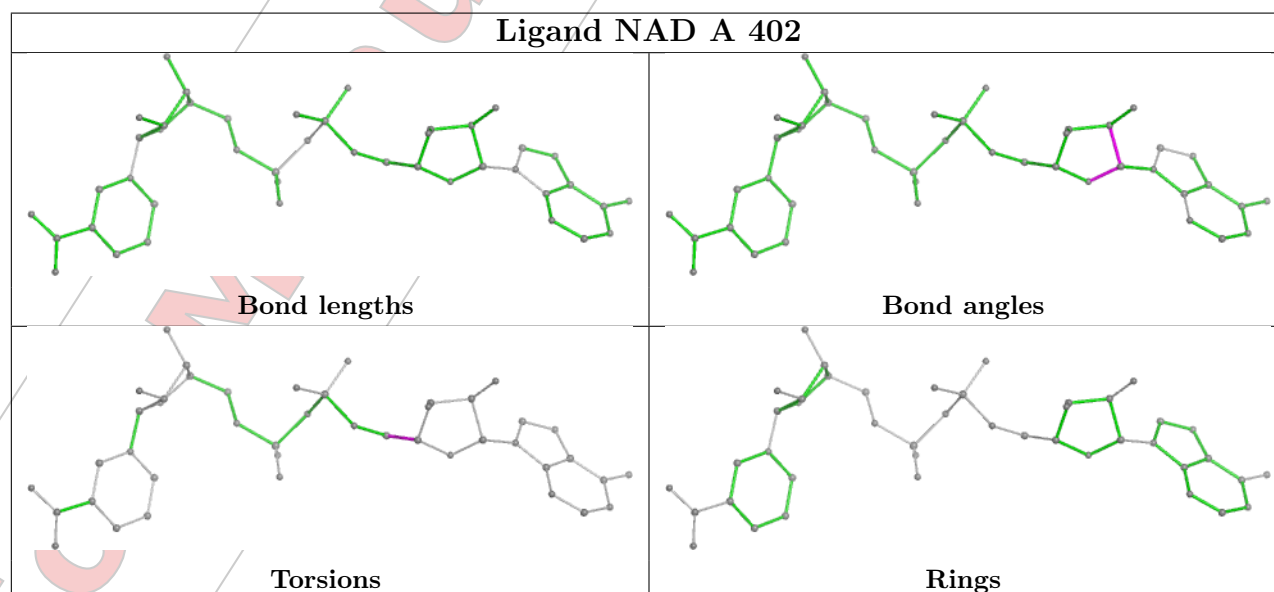
All (11) torsion outliers are listed below:

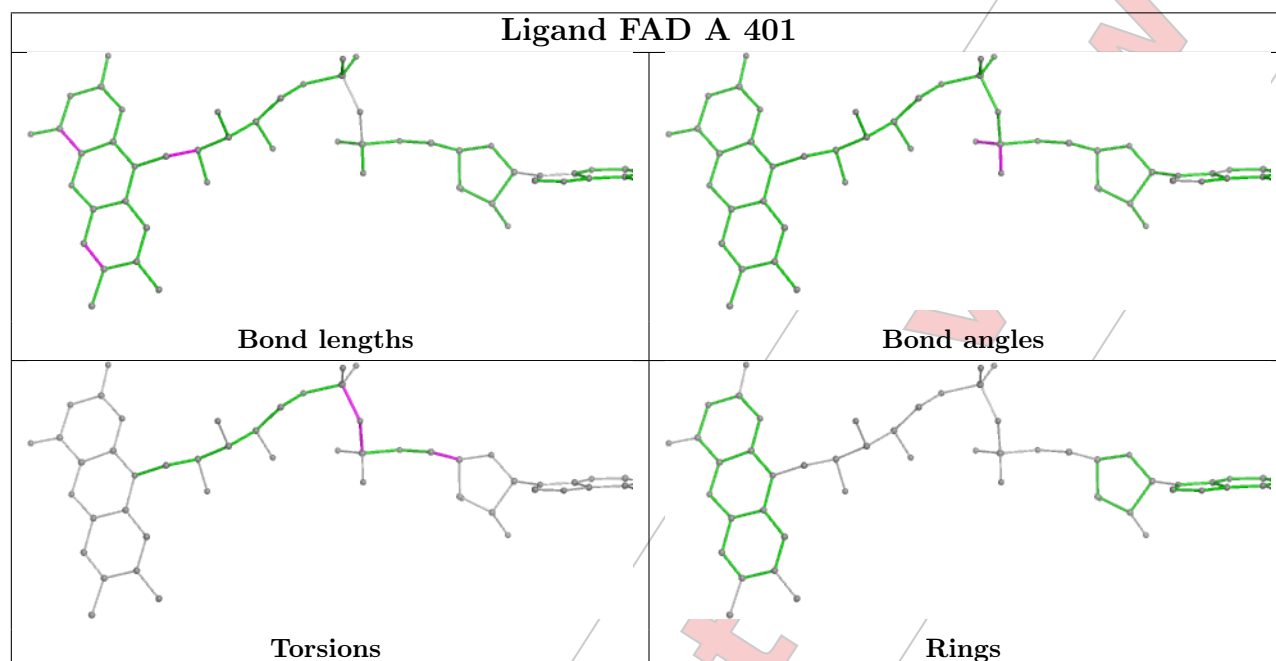
Mol	Chain	Res	Type	Atoms
2	A	401	FAD	PA-O3P-P-O5'
4	A	403	MPD	C2-C3-C4-O4
5	A	404	GOL	O1-C1-C2-C3
5	A	404	GOL	O1-C1-C2-O2
4	A	403	MPD	C1-C2-C3-C4
2	A	401	FAD	P-O3P-PA-O1A
4	A	403	MPD	O2-C2-C3-C4
2	A	401	FAD	O4B-C4B-C5B-O5B
2	A	401	FAD	P-O3P-PA-O2A
4	A	403	MPD	C2-C3-C4-C5
3	A	402	NAD	O4B-C4B-C5B-O5B

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 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, 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	364/368 (98%)	0.19	14 (3%)	44 45	6, 20, 37, 56	6 (1%)

All (14) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	117	VAL	3.8
1	A	367	PRO	3.5
1	A	361	GLU	3.3
1	A	82	GLU	3.1
1	A	357	LYS	2.8
1	A	360	LYS	2.8
1	A	356	GLY	2.7
1	A	365	THR	2.6
1	A	116	PRO	2.5
1	A	4	ASP	2.4
1	A	5	SER	2.3
1	A	69	ASP	2.3
1	A	207	GLN	2.3
1	A	118	SER	2.2

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](#)

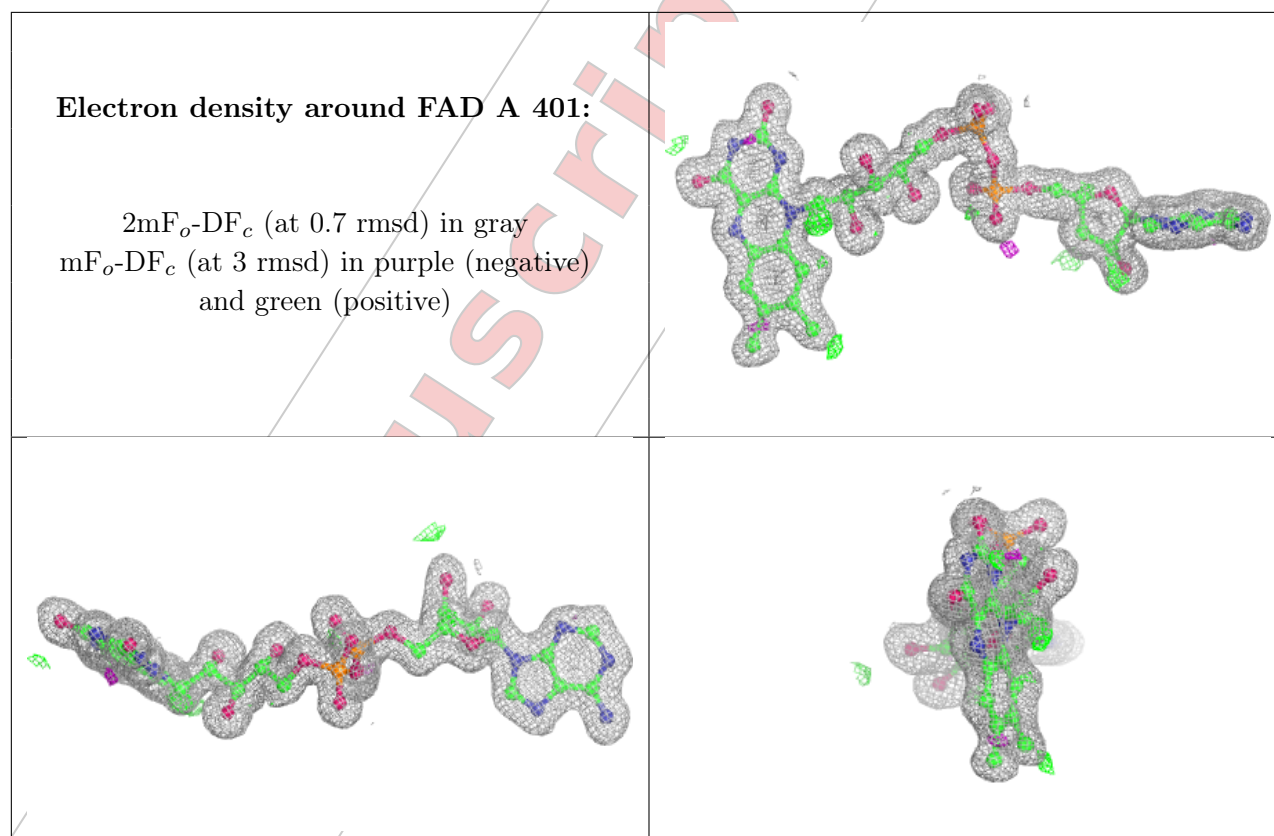
There are no monosaccharides in this entry.

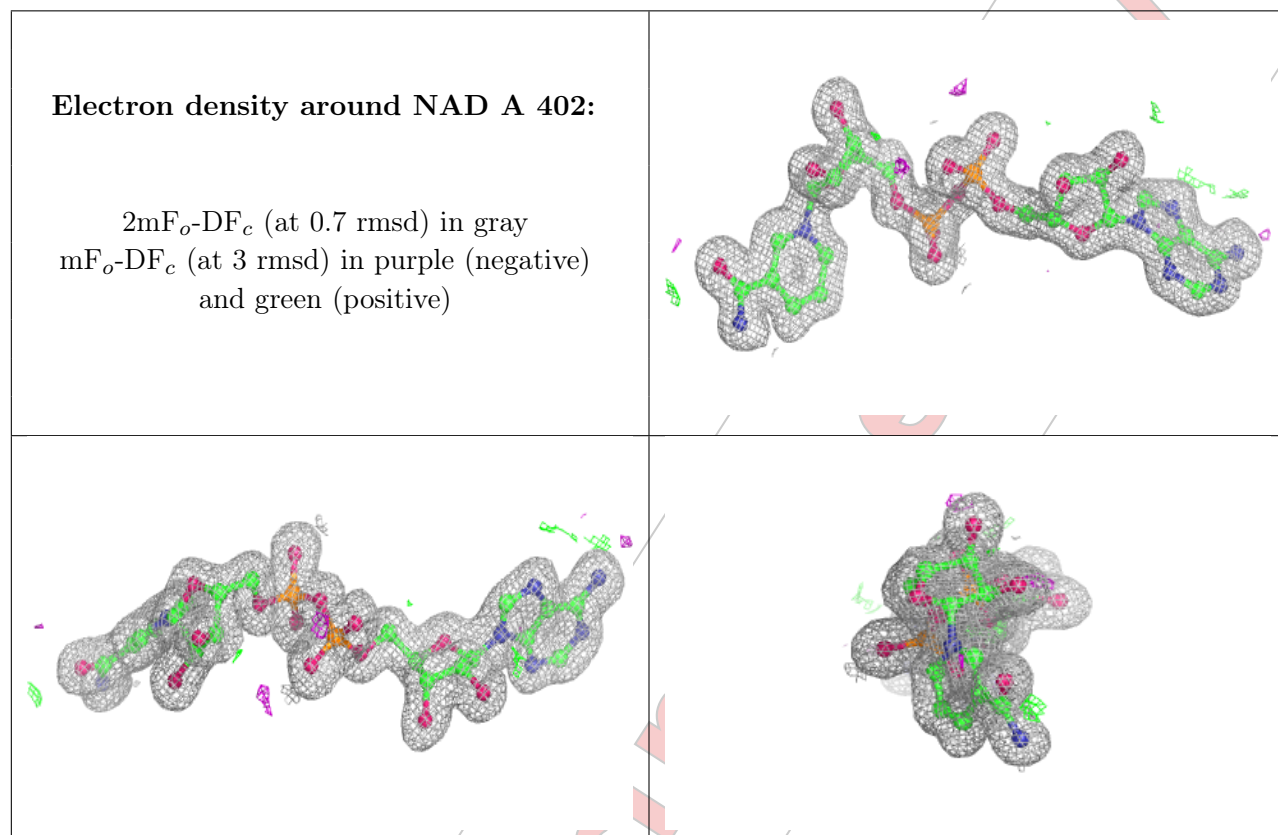
6.4 Ligands ⓘ

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	GOL	A	404	6/6	0.76	0.19	35,39,43,52	0
4	MPD	A	403	8/8	0.83	0.18	34,38,43,44	0
2	FAD	A	401	53/53	0.98	0.05	11,14,19,23	0
3	NAD	A	402	44/44	0.99	0.04	12,15,18,22	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.





6.5 Other polymers [i](#)

There are no such residues in this entry.



Full wwPDB X-ray Structure Validation Report ⓘ

Feb 19, 2025 – 10:13 am GMT

PDB ID : 9IFU
Title : FSP1 (tetrapod ancestor) bound to FAD and NAD⁺ and compound 1
Deposited on : 2025-02-18
Resolution : 1.55 Å (reported)

This wwPDB validation report is for manuscript review

This is a Full wwPDB X-ray Structure Validation Report.

This report is produced by the wwPDB biocuration pipeline after annotation of the structure.

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 ⓘ symbol.

The types of validation reports are described at

<https://www.wwpdb.org/validation/2017/FAQs#types>.

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

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Density-Fitness	:	1.0.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)

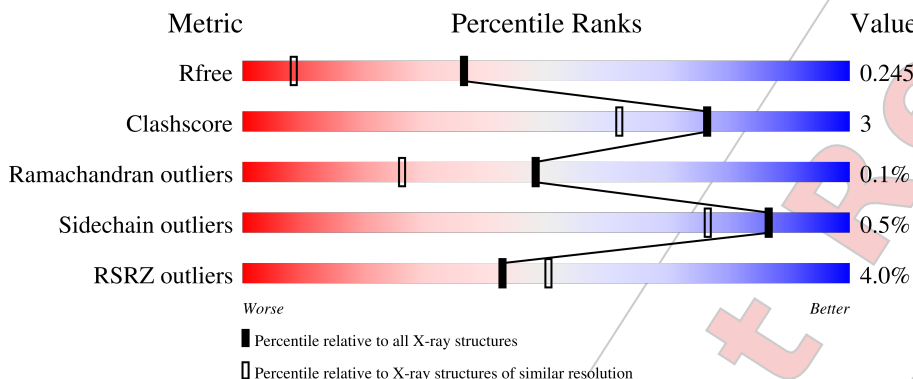
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

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Mol	Chain	Length	Quality of chain
1	A	368	93% 6%
1	B	368	7% 92% 8%

2 Entry composition [i](#)

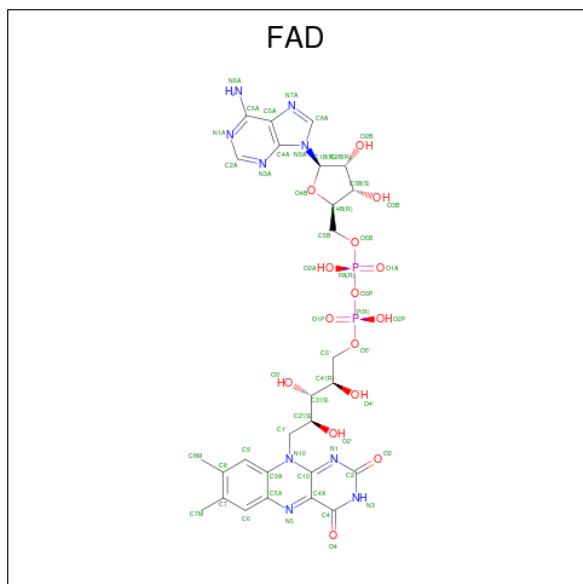
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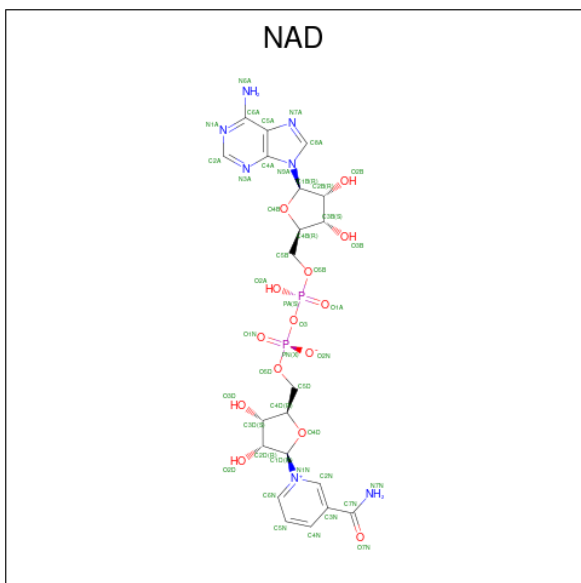
In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

- Molecule 1 is a protein called FSP1 (tetrapod ancestor).

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	365	Total	C	N	O	S	0	8	0
			2827	1789	485	539	14			
1	B	365	Total	C	N	O	S	0	2	0
			2784	1765	476	529	14			

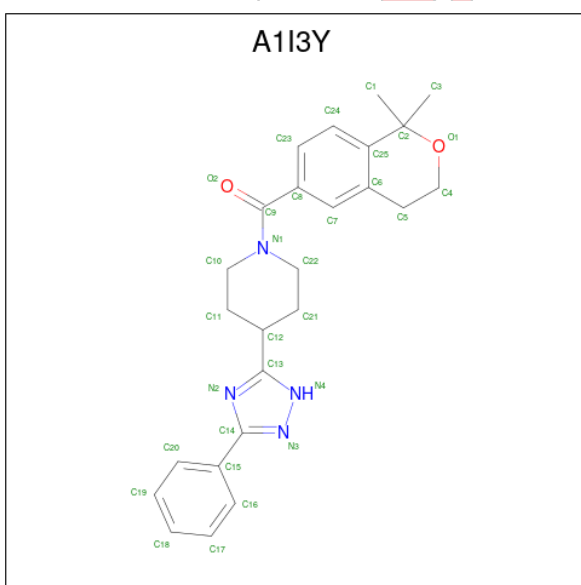
- Molecule 2 is FLAVIN-ADENINE DINUCLEOTIDE (three-letter code: FAD) (formula: $C_{27}H_{33}N_9O_{15}P_2$).





Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	
3	A	1	Total	C	N	O	P	0	0
			44	21	7	14	2		
3	B	1	Total	C	N	O	P	0	0
			44	21	7	14	2		

- Molecule 4 is (1,1-dimethyl-3,4-dihydroisochromen-6-yl)-[4-(3-phenyl-1 {H}-1,2,4-triazol-5-yl)piperidin-1-yl]methanone (three-letter code: A1I3Y) (formula: C₂₅H₂₈N₄O₂) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
4	A	1	Total	C	N	O	0	0
			31	25	4	2		

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Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
4	B	1	Total	C	N	O	0	0
			31	25	4	2		

- Molecule 5 is CHLORIDE ION (three-letter code: CL) (formula: Cl).

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

- Molecule 6 is MAGNESIUM ION (three-letter code: MG) (formula: Mg).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
6	B	1	Total	Mg	0	0
			1	1		

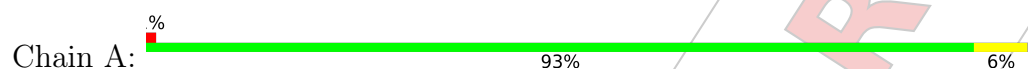
- Molecule 7 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
7	A	366	Total	O	0	0
			366	366		
7	B	190	Total	O	0	0
			190	190		

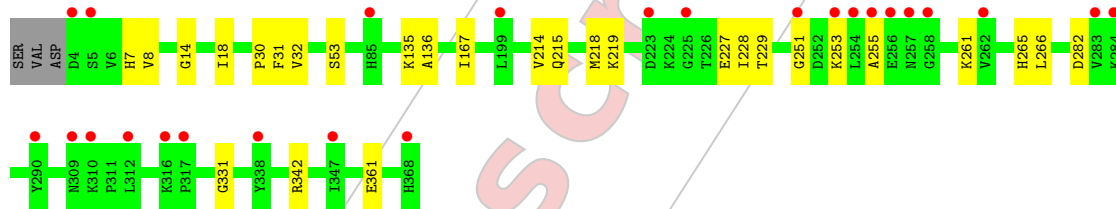
3 Residue-property plots [i](#)

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: FSP1 (tetrapod ancestor)



- Molecule 1: FSP1 (tetrapod ancestor)



4 Data and refinement statistics

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α , β , γ	43.57Å 79.68Å 113.06Å 90.00° 98.61° 90.00°	Depositor
Resolution (Å)	64.97 – 1.55 64.97 – 1.55	Depositor EDS
% Data completeness (in resolution range)	42.5 (64.97-1.55) 42.5 (64.97-1.55)	Depositor EDS
R_{merge}	0.11	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.51 (at 1.55Å)	Xtriage
Refinement program	REFMAC 5.8.0405	Depositor
R, R_{free}	0.179 , 0.242 0.188 , 0.245	Depositor DCC
R_{free} test set	5485 reflections (4.85%)	wwPDB-VP
Wilson B-factor (Å ²)	15.5	Xtriage
Anisotropy	0.179	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.33 , 32.5	EDS
L-test for twinning ²	$\langle L \rangle = 0.50$, $\langle L^2 \rangle = 0.33$	Xtriage
Estimated twinning fraction	0.031 for h,-k,-h-l	Xtriage
F_o, F_c correlation	0.94	EDS
Total number of atoms	6425	wwPDB-VP
Average B, all atoms (Å ²)	22.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.17% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical 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: MG, FAD, CL, A1I3Y, NAD

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	0.39	0/2876	0.69	0/3882
1	B	0.36	0/2832	0.66	0/3824
All	All	0.38	0/5708	0.68	0/7706

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	1

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	202	ARG	Sidechain

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	2827	0	2844	14	0
1	B	2784	0	2810	18	0
2	A	53	0	31	0	0
2	B	53	0	31	0	0
3	A	44	0	26	1	0
3	B	44	0	26	0	0
4	A	31	0	0	3	0
4	B	31	0	0	4	0
5	A	1	0	0	0	0
6	B	1	0	0	0	0
7	A	366	0	0	0	1
7	B	190	0	0	0	0
All	All	6425	0	5768	33	1

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 3.

All (33) 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:7[A]:HIS:HE1	1:A:32:VAL:HG23	1.24	1.01
1:A:7[A]:HIS:CE1	1:A:32:VAL:HG23	2.00	0.97
1:B:7:HIS:HE1	1:B:32:VAL:HG23	1.45	0.81
1:A:58:LYS:HE2	1:A:368:HIS:HB2	1.72	0.72
1:B:219:LYS:NZ	1:B:227:GLU:OE1	2.26	0.68
1:B:7:HIS:CE1	1:B:32:VAL:HG23	2.33	0.60
1:B:14:GLY:O	1:B:18:ILE:HG12	2.08	0.54
1:A:146[A]:SER:OG	1:A:175:ASP:OD1	2.15	0.53
1:B:167:ILE:HD11	1:B:228:ILE:HD11	1.93	0.49
1:B:361:GLU:HG2	4:B:403:A1I3Y:C19	2.44	0.48
1:A:334:GLN:HA	1:A:338:TYR:O	2.14	0.47
1:B:361:GLU:HG2	4:B:403:A1I3Y:C18	2.46	0.45
1:A:361:GLU:HG2	4:A:403:A1I3Y:C17	2.47	0.45
1:B:135:LYS:HB2	1:B:214:VAL:HG21	1.99	0.44
1:A:361:GLU:HG2	4:A:403:A1I3Y:C18	2.47	0.44
1:B:265:HIS:O	1:B:266:LEU:HB2	2.17	0.44
1:B:136:ALA:HB2	1:B:214:VAL:HG22	1.99	0.44
1:B:18:ILE:HD11	4:B:403:A1I3Y:C19	2.48	0.44
1:B:7:HIS:CD2	1:B:30:PRO:HB2	2.53	0.43
1:B:331:GLY:O	1:B:342:ARG:HG2	2.18	0.43
1:A:42:HIS:NE2	1:A:44:VAL:CG2	2.81	0.43
1:A:18:ILE:HD12	1:A:40:PHE:CD1	2.53	0.43

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
4:B:403:A1I3Y:C7	4:B:403:A1I3Y:C22	2.97	0.43
1:B:255:ALA:HB2	1:B:282:ASP:OD2	2.19	0.42
1:B:218:MET:O	1:B:229:THR:HA	2.20	0.42
1:A:53[A]:SER:HB3	1:A:130:VAL:HG11	2.01	0.42
1:A:287:LYS:O	3:A:402:NAD:H1D	2.18	0.42
1:B:361:GLU:O	1:B:361:GLU:HG3	2.19	0.41
1:A:331:GLY:O	1:A:342:ARG:HA	2.20	0.41
1:B:253:LYS:O	1:B:261:LYS:HG2	2.21	0.40
1:A:193:LYS:HE3	1:A:326:MET:O	2.21	0.40
1:A:290:TYR:CD1	4:A:403:A1I3Y:C23	3.04	0.40
1:B:8:VAL:O	1:B:31:PHE:HA	2.22	0.40

All (1) symmetry-related close contacts are listed below. The label for Atom-2 includes the symmetry operator and encoded unit-cell translations to be applied.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
7:A:740:HOH:O	7:A:806:HOH:O[2_544]	1.97	0.23

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	371/368 (101%)	363 (98%)	8 (2%)	0	100	100
1	B	365/368 (99%)	357 (98%)	7 (2%)	1 (0%)	37	18
All	All	736/736 (100%)	720 (98%)	15 (2%)	1 (0%)	48	26

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	B	251	GLY

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	307/302 (102%)	306 (100%)	1 (0%)	91	84
1	B	301/302 (100%)	299 (99%)	2 (1%)	81	67
All	All	608/604 (101%)	605 (100%)	3 (0%)	86	76

All (3) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	297	ASN
1	B	53	SER
1	B	215	GLN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
1	B	7	HIS

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

Of 8 ligands modelled in this entry, 2 are 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
3	NAD	B	402	-	42,48,48	0.70	1 (2%)	50,73,73	0.80	1 (2%)
2	FAD	B	401	-	53,58,58	0.66	0	68,89,89	0.86	3 (4%)
4	A1I3Y	B	403	-	31,35,35	0.54	0	47,51,51	0.82	2 (4%)
4	A1I3Y	A	403	-	31,35,35	0.53	0	47,51,51	0.63	0
3	NAD	A	402	-	42,48,48	0.72	2 (4%)	50,73,73	0.83	1 (2%)
2	FAD	A	401	-	53,58,58	0.66	0	68,89,89	0.87	2 (2%)

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	NAD	B	402	-	-	1/26/62/62	0/5/5/5
2	FAD	B	401	-	-	8/30/50/50	0/6/6/6
4	A1I3Y	B	403	-	-	0/12/39/39	1/5/5/5
4	A1I3Y	A	403	-	-	2/12/39/39	1/5/5/5
3	NAD	A	402	-	-	1/26/62/62	0/5/5/5
2	FAD	A	401	-	-	5/30/50/50	0/6/6/6

All (3) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	A	402	NAD	C2N-N1N	2.49	1.38	1.35
3	B	402	NAD	C2N-N1N	2.13	1.37	1.35
3	A	402	NAD	C8A-N7A	-2.12	1.30	1.34

All (9) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
3	A	402	NAD	C6N-N1N-C2N	-2.43	119.76	121.97
2	A	401	FAD	C5A-C6A-N6A	2.28	123.82	120.35
4	B	403	A1I3Y	C4-C5-C6	2.28	114.14	110.87
2	A	401	FAD	C4-N3-C2	-2.18	121.61	125.64
4	B	403	A1I3Y	C11-C10-N1	2.18	114.14	110.82
3	B	402	NAD	C6N-N1N-C2N	-2.08	120.07	121.97
2	B	401	FAD	C4-N3-C2	-2.08	121.79	125.64
2	B	401	FAD	O4-C4-C4X	-2.07	121.11	126.60
2	B	401	FAD	O4B-C1B-C2B	-2.06	103.92	106.93

There are no chirality outliers.

All (17) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	A	401	FAD	O2'-C2'-C3'-O3'
2	B	401	FAD	O3'-C3'-C4'-C5'
2	B	401	FAD	PA-O3P-P-O5'
2	B	401	FAD	O3'-C3'-C4'-O4'
2	B	401	FAD	O2'-C2'-C3'-O3'
2	A	401	FAD	O4B-C4B-C5B-O5B
2	A	401	FAD	O2'-C2'-C3'-C4'
2	A	401	FAD	P-O3P-PA-O2A
2	B	401	FAD	P-O3P-PA-O1A
4	A	403	A1I3Y	N2-C14-C15-C20
2	B	401	FAD	O4B-C4B-C5B-O5B
3	B	402	NAD	O4B-C4B-C5B-O5B
2	B	401	FAD	P-O3P-PA-O2A
3	A	402	NAD	O4B-C4B-C5B-O5B
2	A	401	FAD	C1'-C2'-C3'-O3'
2	B	401	FAD	C1'-C2'-C3'-O3'
4	A	403	A1I3Y	N2-C14-C15-C16

All (2) ring outliers are listed below:

Mol	Chain	Res	Type	Atoms
4	B	403	A1I3Y	C10-C11-C12-C21-C22-N1
4	A	403	A1I3Y	C10-C11-C12-C21-C22-N1

3 monomers are involved in 8 short contacts:

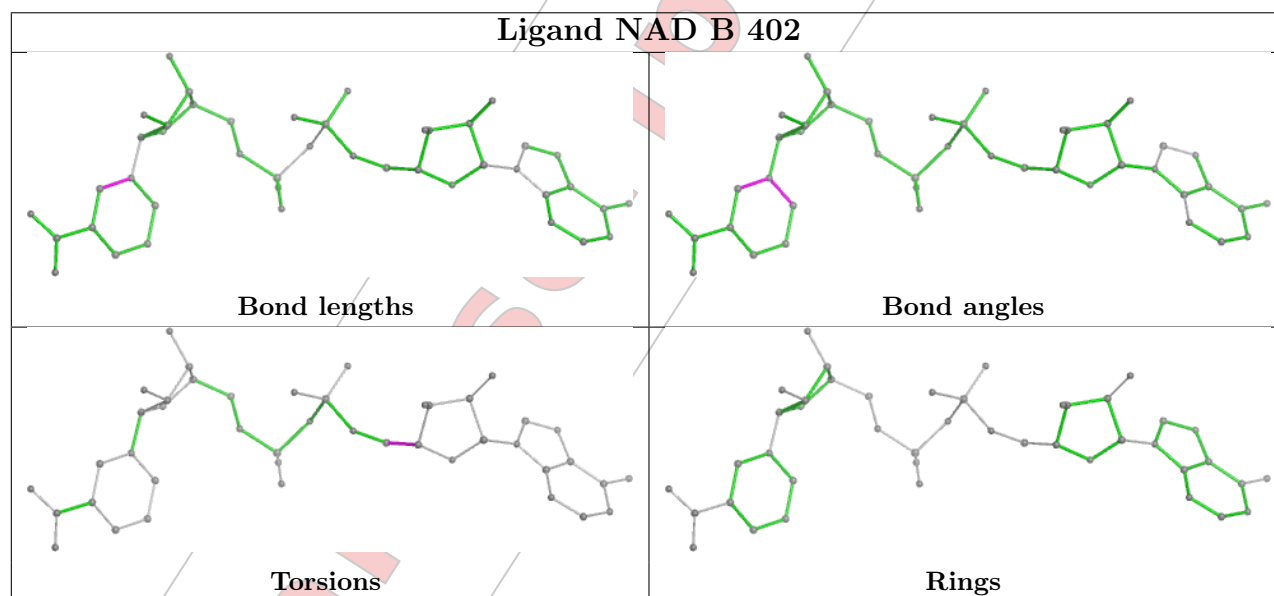
Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	B	403	A1I3Y	4	0

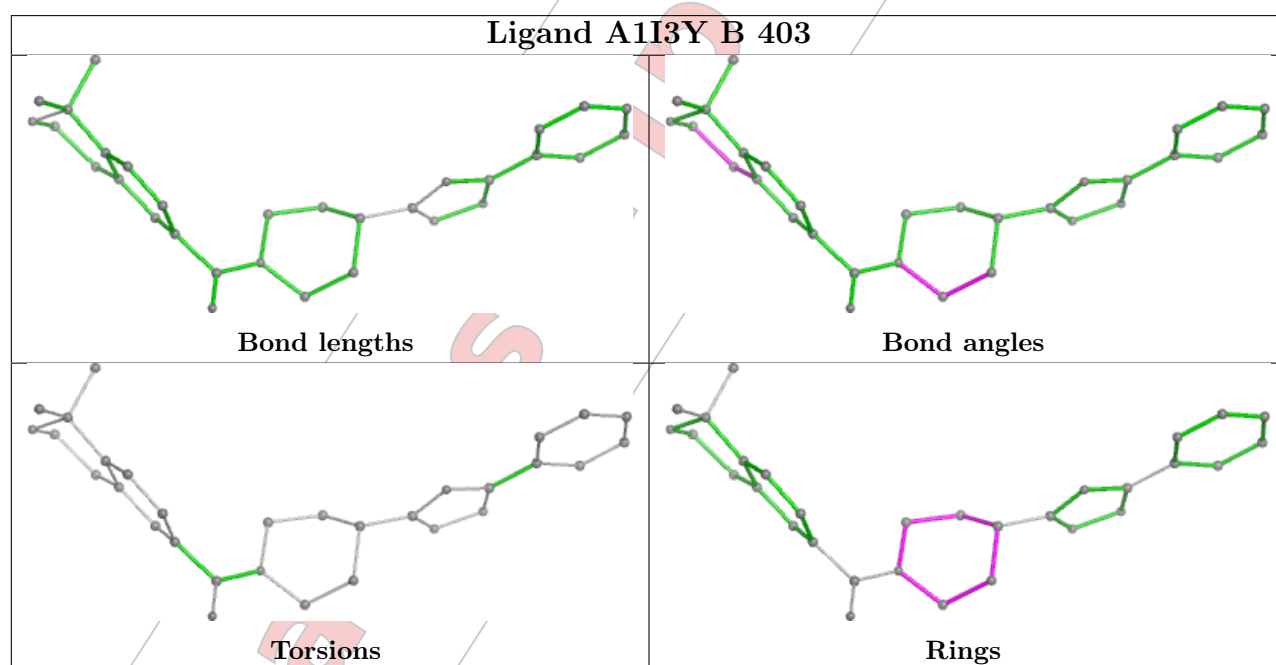
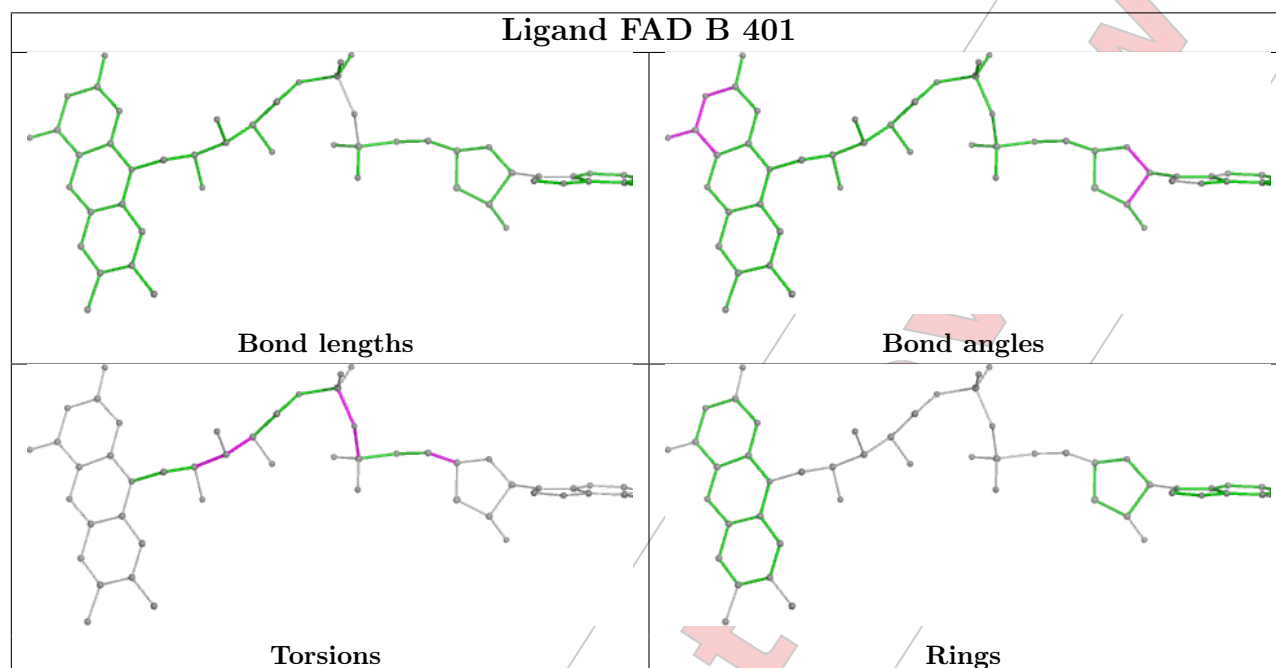
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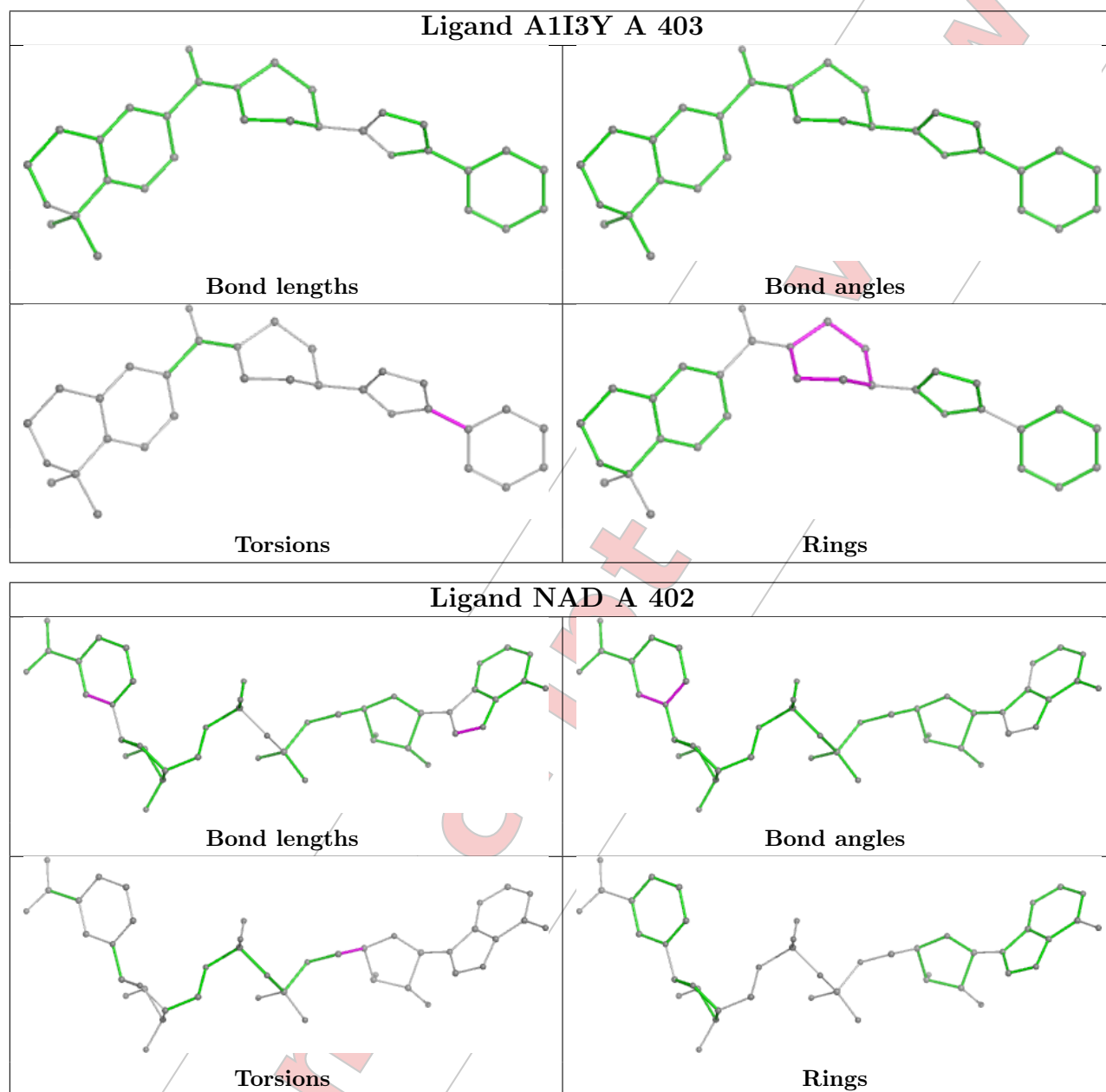
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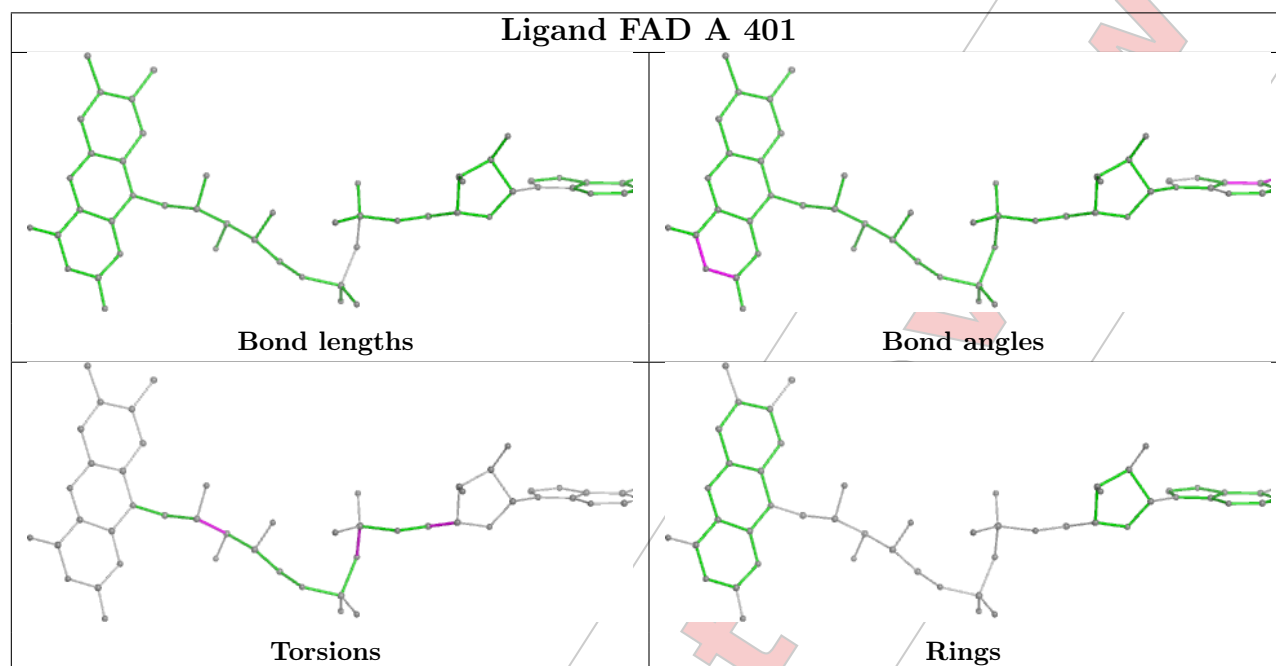
Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	A	403	A1I3Y	3	0
3	A	402	NAD	1	0

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

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	365/368 (99%)	0.02	4 (1%) 77 84	4, 14, 32, 85	8 (2%)
1	B	365/368 (99%)	0.54	25 (6%) 25 29	7, 23, 48, 66	2 (0%)
All	All	730/736 (99%)	0.28	29 (3%) 43 50	4, 18, 44, 85	10 (1%)

All (29) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	368	HIS	6.4
1	B	255	ALA	4.9
1	B	251	GLY	3.4
1	B	310	LYS	3.3
1	B	283	VAL	3.1
1	A	7[A]	HIS	2.8
1	B	309	ASN	2.7
1	B	316	LYS	2.7
1	B	254	LEU	2.7
1	B	290	TYR	2.6
1	B	257	ASN	2.5
1	B	85	HIS	2.5
1	A	5	SER	2.5
1	B	338	TYR	2.5
1	B	258	GLY	2.4
1	B	4	ASP	2.4
1	B	5	SER	2.3
1	B	256	GLU	2.3
1	B	317	PRO	2.2
1	B	368	HIS	2.2
1	B	223	ASP	2.2
1	B	253	LYS	2.2
1	B	225	GLY	2.1
1	A	227	GLU	2.1

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Mol	Chain	Res	Type	RSRZ
1	B	199	LEU	2.1
1	B	262	VAL	2.1
1	B	284	LYS	2.1
1	B	312	LEU	2.0
1	B	347	ILE	2.0

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](#)

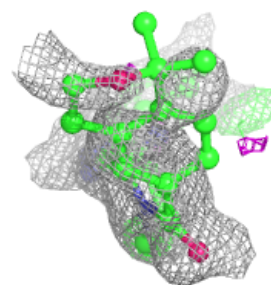
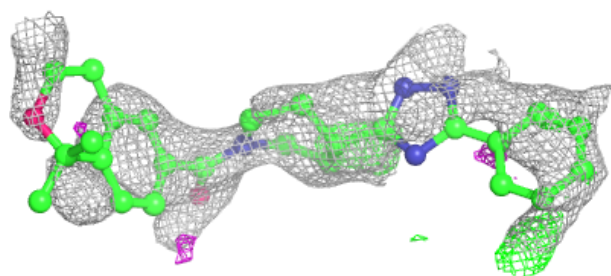
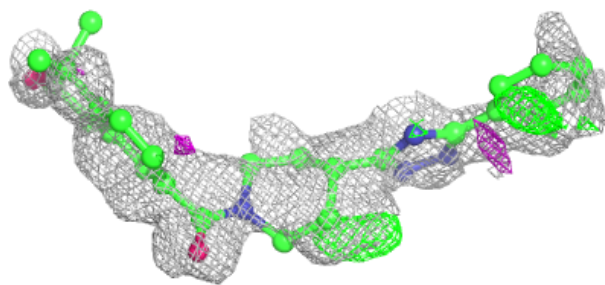
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95th percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists 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	A1I3Y	B	403	31/31	0.73	0.22	50,70,90,92	0
4	A1I3Y	A	403	31/31	0.79	0.21	32,51,67,68	0
5	CL	A	404	1/1	0.90	0.17	61,61,61,61	0
6	MG	B	404	1/1	0.93	0.09	31,31,31,31	0
3	NAD	B	402	44/44	0.96	0.06	12,16,19,24	0
2	FAD	B	401	53/53	0.97	0.05	11,12,17,17	0
2	FAD	A	401	53/53	0.97	0.05	7,8,11,13	0
3	NAD	A	402	44/44	0.98	0.04	6,9,14,14	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.

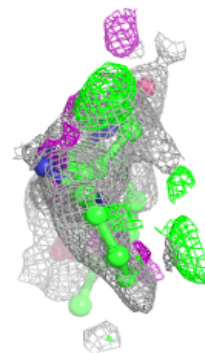
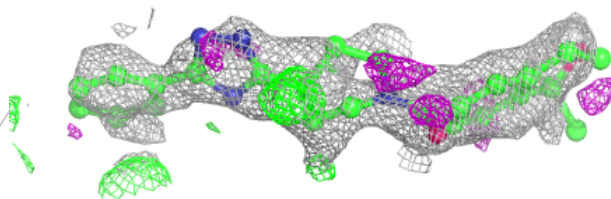
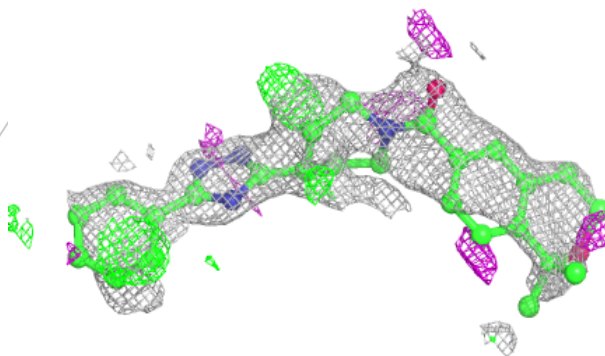
Electron density around A1I3Y B 403:

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



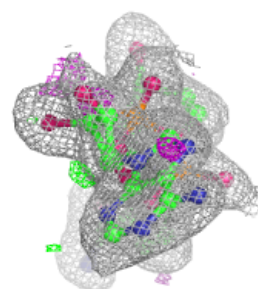
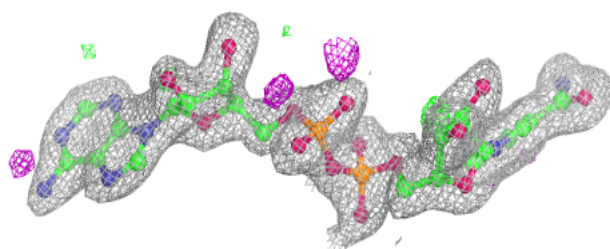
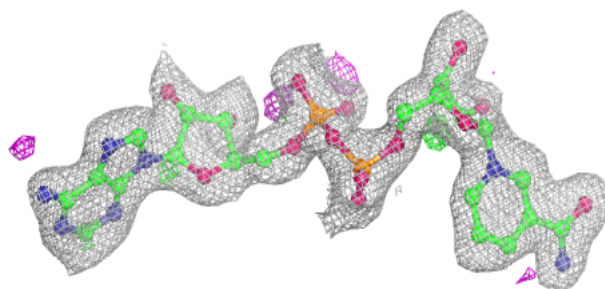
Electron density around A1I3Y A 403:

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



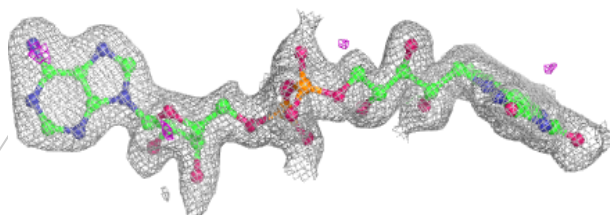
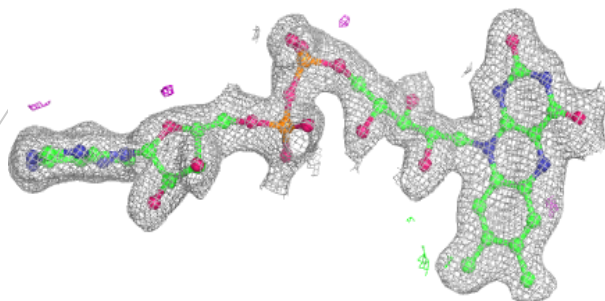
Electron density around NAD B 402:

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



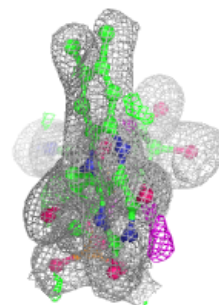
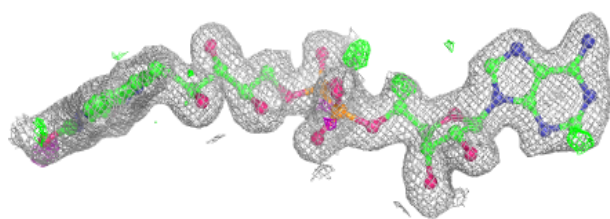
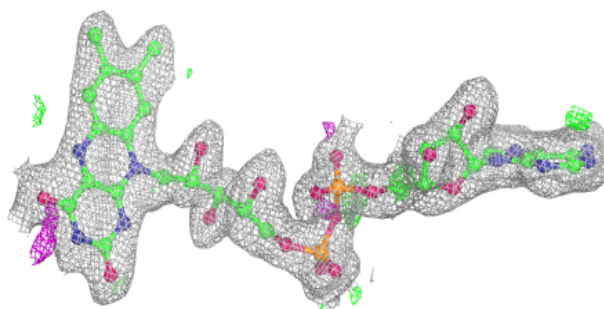
Electron density around FAD B 401:

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



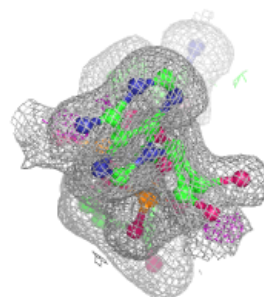
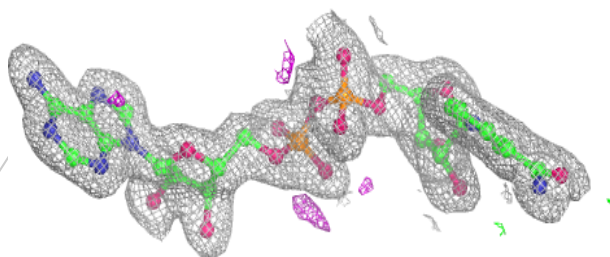
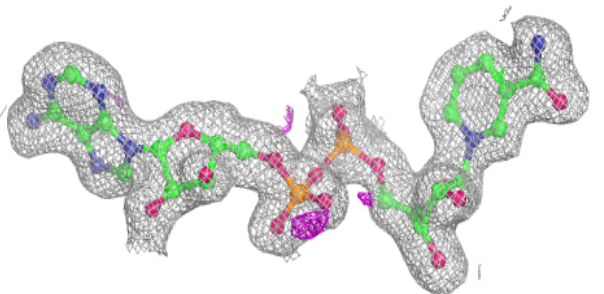
Electron density around FAD A 401:

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



Electron density around NAD A 402:

$2mF_o-DF_c$ (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.

For Manuscript Review



Full wwPDB X-ray Structure Validation Report ⓘ

Feb 19, 2025 – 10:14 am GMT

PDB ID : 9IFW
Title : FSP1 (tetrapod ancestor) bound to FAD and NAD⁺ and compound 4
Deposited on : 2025-02-18
Resolution : 1.74 Å (reported)

This wwPDB validation report is for manuscript review

This is a Full wwPDB X-ray Structure Validation Report.

This report is produced by the wwPDB biocuration pipeline after annotation of the structure.

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 ⓘ symbol.

The types of validation reports are described at

<https://www.wwpdb.org/validation/2017/FAQs#types>.

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

MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (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	:	9.0.003 (Gargrove)
Density-Fitness	:	1.0.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)

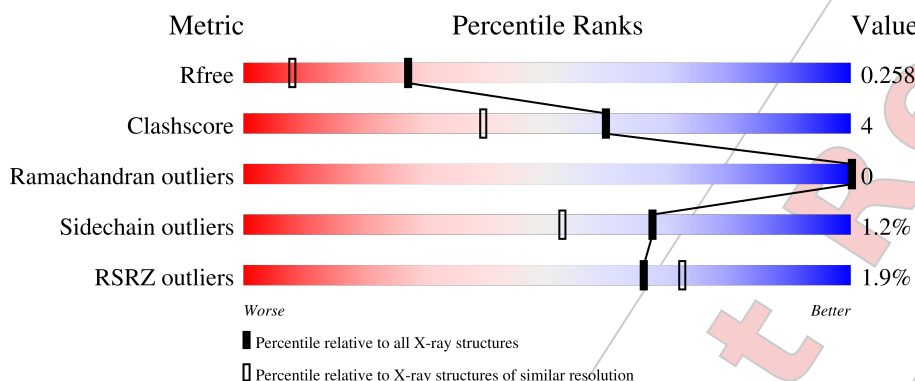
1 Overall quality at a glance i

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 1.74 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	1043 (1.74-1.74)
Clashscore	180529	1119 (1.74-1.74)
Ramachandran outliers	177936	1112 (1.74-1.74)
Sidechain outliers	177891	1112 (1.74-1.74)
RSRZ outliers	164620	1043 (1.74-1.74)

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 $\leq 5\%$. 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	368	90% 8% ..
1	B	368	3% 90% 9% .

2 Entry composition [i](#)

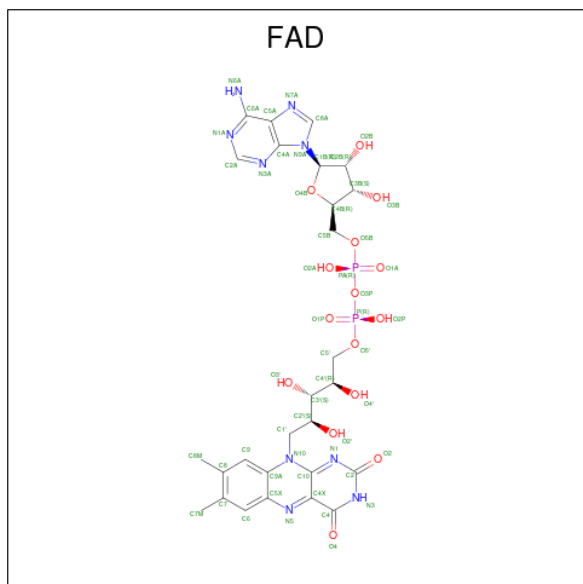
There are 10 unique types of molecules in this entry. The entry contains 6112 atoms, of which 0 are hydrogens and 0 are deuteriums.

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 FSP1 (Tetrapod ancestor).

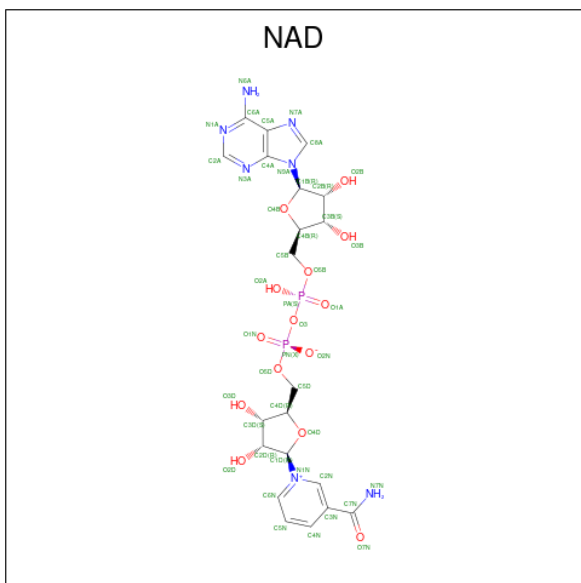
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	364	Total	C	N	O	S	0	2	0
			2777	1762	474	527	14			
1	B	364	Total	C	N	O	S	0	3	0
			2783	1765	475	529	14			

- Molecule 2 is FLAVIN-ADENINE DINUCLEOTIDE (three-letter code: FAD) (formula: $C_{27}H_{33}N_9O_{15}P_2$).



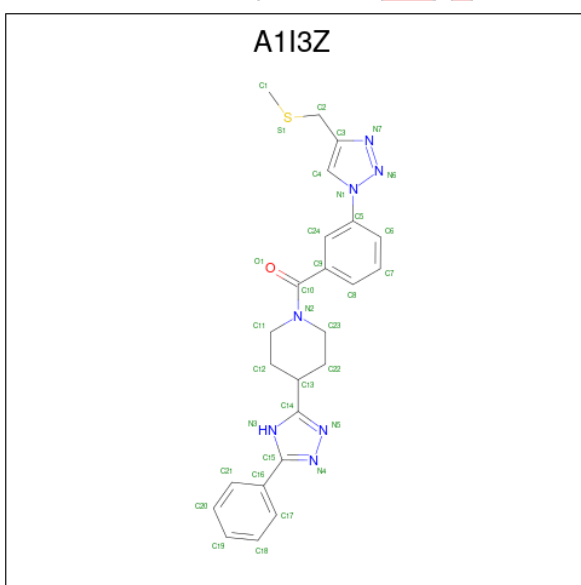
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
2	A	1	Total	C	N	O	P	0	0
			53	27	9	15	2		
2	B	1	Total	C	N	O	P	0	0
			53	27	9	15	2		

- Molecule 3 is NICOTINAMIDE-ADENINE-DINUCLEOTIDE (three-letter code: NAD) (formula: $C_{21}H_{27}N_7O_{14}P_2$).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	
3	A	1	Total	C	N	O	P	0	0
			44	21	7	14	2		
3	B	1	Total	C	N	O	P	0	0
			44	21	7	14	2		

- Molecule 4 is [3-[4-(methylsulfanylmethyl)-1,2,3-triazol-1-yl]phenyl]-[4-(5-phenyl-4 {H}-1,2,4-triazol-3-yl)piperidin-1-yl]methanone (three-letter code: A1I3Z) (formula: C₂₄H₂₅N₇OS) (labeled as "Ligand of Interest" by depositor).



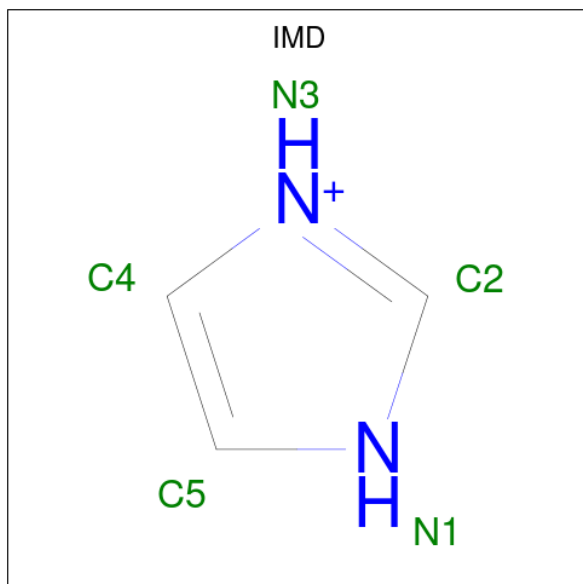
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
4	A	1	Total	C	N	O	S	0	0
			33	24	7	1	1		

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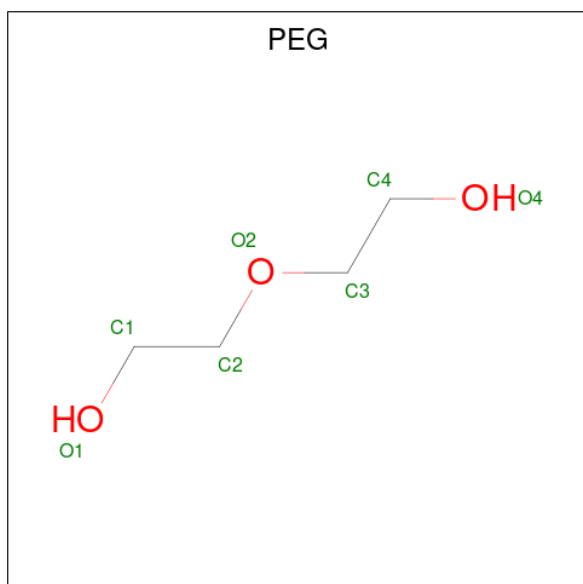
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
			Total	C	N	O	S		
4	B	1	33	24	7	1	1	0	0

- Molecule 5 is IMIDAZOLE (three-letter code: IMD) (formula: $C_3H_5N_2$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
			Total	C	N		
5	A	1	5	3	2	0	0

- Molecule 6 is DI(HYDROXYETHYL)ETHER (three-letter code: PEG) (formula: $C_4H_{10}O_3$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
6	A	1	Total	C	O	0	0
			7	4	3		

- Molecule 7 is CALCIUM ION (three-letter code: CA) (formula: Ca).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
7	A	1	Total	Ca	0	0
			1	1		
7	B	4	Total	Ca	0	0
			4	4		

- Molecule 8 is CHLORIDE ION (three-letter code: CL) (formula: Cl).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
8	A	1	Total	Cl	0	0
			1	1		

- Molecule 9 is MAGNESIUM ION (three-letter code: MG) (formula: Mg).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
9	A	1	Total	Mg	0	0
			1	1		

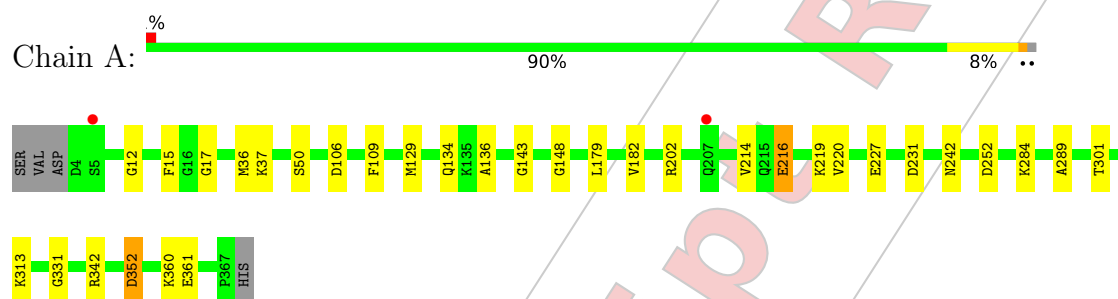
- Molecule 10 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
10	A	183	Total	O	0	0
			183	183		
10	B	90	Total	O	0	0
			90	90		

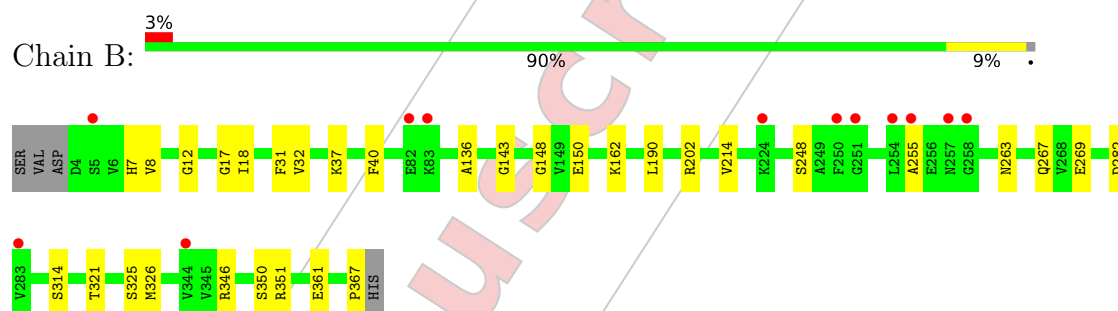
3 Residue-property plots [i](#)

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: FSP1 (Tetrapod ancestor)



- Molecule 1: FSP1 (Tetrapod ancestor)



4 Data and refinement statistics

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α , β , γ	43.66Å 79.51Å 112.78Å 90.00° 98.79° 90.00°	Depositor
Resolution (Å)	42.52 – 1.74 42.52 – 1.74	Depositor EDS
% Data completeness (in resolution range)	96.6 (42.52-1.74) 96.6 (42.52-1.74)	Depositor EDS
R_{merge}	0.10	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.11 (at 1.74Å)	Xtriage
Refinement program	REFMAC 5.8.0405	Depositor
R, R_{free}	0.205 , 0.248 0.215 , 0.258	Depositor DCC
R_{free} test set	3900 reflections (5.01%)	wwPDB-VP
Wilson B-factor (Å ²)	18.7	Xtriage
Anisotropy	0.128	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.35 , 31.1	EDS
L-test for twinning ²	$\langle L \rangle = 0.47$, $\langle L^2 \rangle = 0.29$	Xtriage
Estimated twinning fraction	0.051 for h,-k,-h-l	Xtriage
F_o, F_c correlation	0.95	EDS
Total number of atoms	6112	wwPDB-VP
Average B, all atoms (Å ²)	26.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.60% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical 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: A1I3Z, FAD, MG, NAD, CL, CA, PEG, IMD

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	0.46	0/2824	0.77	0/3812
1	B	0.39	0/2830	0.74	0/3820
All	All	0.43	0/5654	0.75	0/7632

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	1
1	B	0	2
All	All	0	3

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (3) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	202	ARG	Sidechain
1	B	202	ARG	Sidechain
1	B	351	ARG	Sidechain

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	2777	0	2811	28	0
1	B	2783	0	2815	19	0
2	A	53	0	31	5	0
2	B	53	0	31	0	0
3	A	44	0	26	0	0
3	B	44	0	26	0	0
4	A	33	0	0	1	0
4	B	33	0	0	2	0
5	A	5	0	5	0	0
6	A	7	0	10	2	0
7	A	1	0	0	0	0
7	B	4	0	0	0	0
8	A	1	0	0	0	0
9	A	1	0	0	0	0
10	A	183	0	0	5	0
10	B	90	0	0	1	0
All	All	6112	0	5755	49	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.

All (49) 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:50:SER:HB2	1:A:129:MET:HG2	1.59	0.84
1:A:109:PHE:CE2	1:A:129:MET:HE3	2.18	0.79
1:A:109:PHE:CE2	1:A:129:MET:CE	2.70	0.75
1:B:7:HIS:NE2	1:B:32:VAL:HG23	2.09	0.68
1:A:109:PHE:CD2	1:A:129:MET:CE	2.79	0.65
1:A:109:PHE:HE2	1:A:129:MET:HE3	1.64	0.63
1:A:36:MET:HE2	2:A:401:FAD:C6A	2.29	0.62
1:A:36:MET:CE	2:A:401:FAD:C5A	2.76	0.62
1:A:216:GLU:H	1:A:216:GLU:CD	2.05	0.60
1:A:352:ASP:HB3	10:A:662:HOH:O	2.04	0.58
1:B:367:PRO:C	10:B:549:HOH:O	2.42	0.57
1:A:109:PHE:HE2	1:A:129:MET:CE	2.16	0.57
1:A:36:MET:HE3	2:A:401:FAD:C5A	2.34	0.57
1:B:136:ALA:HB2	1:B:214:VAL:HG22	1.88	0.56
1:A:361:GLU:HG2	4:A:403:A1I3Z:C19	2.37	0.55
1:A:36:MET:HE2	2:A:401:FAD:C5A	2.37	0.55

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:B:263:ASN:HD21	1:B:267:GLN:HB2	1.71	0.55
1:A:37[B]:LYS:HB2	1:A:37[B]:LYS:NZ	2.23	0.54
6:A:405:PEG:O2	10:A:501:HOH:O	2.19	0.53
1:B:18:ILE:HD12	1:B:40:PHE:CD1	2.43	0.52
1:B:255:ALA:HB2	1:B:282:ASP:OD2	2.11	0.51
1:A:136:ALA:HB2	1:A:214:VAL:HG22	1.93	0.50
1:A:36:MET:CE	2:A:401:FAD:C6A	2.90	0.49
1:A:352:ASP:HB2	10:A:565:HOH:O	2.14	0.48
1:B:269:GLU:HA	1:B:269:GLU:OE1	2.13	0.48
1:B:143:GLY:O	1:B:148:GLY:HA3	2.13	0.48
1:B:190:LEU:HD21	1:B:326:MET:HE1	1.94	0.48
1:B:321:THR:HG21	4:B:403:A1I3Z:C7	2.46	0.46
1:A:12:GLY:O	1:A:17:GLY:HA3	2.16	0.46
1:A:106:ASP:HB2	1:A:242:ASN:OD1	2.16	0.46
1:A:134:GLN:HG3	10:A:506:HOH:O	2.16	0.45
1:B:346:ARG:HA	1:B:350:SER:HB2	1.97	0.45
6:A:405:PEG:H41	6:A:405:PEG:H22	1.77	0.45
1:B:18:ILE:CD1	1:B:40:PHE:CD1	3.00	0.45
1:A:331:GLY:O	1:A:342:ARG:HA	2.17	0.44
1:B:37[B]:LYS:NZ	1:B:37[B]:LYS:HB2	2.32	0.44
1:A:179:LEU:HB2	1:A:182:VAL:HG23	1.99	0.43
1:B:136:ALA:O	1:B:162:LYS:NZ	2.41	0.43
1:A:214:VAL:HA	1:A:231:ASP:O	2.18	0.43
1:B:12:GLY:O	1:B:17:GLY:HA3	2.19	0.43
1:A:284:LYS:HG2	10:A:522:HOH:O	2.18	0.42
1:B:255:ALA:CB	1:B:282:ASP:OD2	2.66	0.42
1:A:301:THR:HG21	1:A:313:LYS:HD2	2.01	0.42
1:B:361:GLU:HG2	4:B:403:A1I3Z:C19	2.50	0.41
1:B:150:GLU:HG2	1:B:325:SER:O	2.19	0.41
1:A:143:GLY:O	1:A:148:GLY:HA3	2.21	0.41
1:A:15:PHE:CG	1:A:289:ALA:HB1	2.56	0.41
1:A:220:VAL:O	1:A:227:GLU:HA	2.21	0.41
1:B:8:VAL:O	1:B:31:PHE:HA	2.20	0.41

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	364/368 (99%)	357 (98%)	7 (2%)	0	100	100
1	B	365/368 (99%)	355 (97%)	10 (3%)	0	100	100
All	All	729/736 (99%)	712 (98%)	17 (2%)	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	300/302 (99%)	295 (98%)	5 (2%)	56	34
1	B	301/302 (100%)	299 (99%)	2 (1%)	81	74
All	All	601/604 (100%)	594 (99%)	7 (1%)	67	52

All (7) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	216	GLU
1	A	219	LYS
1	A	252	ASP
1	A	352	ASP
1	A	360	LYS
1	B	248	SER
1	B	314	SER

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
1	B	23	GLN

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](#)

Of 15 ligands modelled in this entry, 7 are monoatomic - leaving 8 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	IMD	A	404	-	3,5,5	0.47	0	4,5,5	0.69	0
2	FAD	B	401	7	53,58,58	0.60	0	68,89,89	0.87	3 (4%)
3	NAD	B	402	-	42,48,48	0.66	1 (2%)	50,73,73	0.85	2 (4%)
3	NAD	A	402	-	42,48,48	0.76	2 (4%)	50,73,73	0.95	2 (4%)
2	FAD	A	401	-	53,58,58	0.88	1 (1%)	68,89,89	0.90	2 (2%)
4	A1I3Z	A	403	-	33,37,37	0.67	0	41,51,51	1.31	8 (19%)
6	PEG	A	405	-	6,6,6	0.53	0	5,5,5	0.45	0
4	A1I3Z	B	403	-	33,37,37	0.64	0	41,51,51	1.23	4 (9%)

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	IMD	A	404	-	-	-	0/1/1/1
2	FAD	B	401	7	-	7/30/50/50	0/6/6/6
3	NAD	B	402	-	-	1/26/62/62	0/5/5/5
3	NAD	A	402	-	-	1/26/62/62	0/5/5/5
2	FAD	A	401	-	-	7/30/50/50	0/6/6/6
4	A1I3Z	A	403	-	-	5/19/33/33	1/5/5/5
6	PEG	A	405	-	-	3/4/4/4	-
4	A1I3Z	B	403	-	-	3/19/33/33	1/5/5/5

All (4) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	401	FAD	C1'-C2'	-3.46	1.47	1.52
3	A	402	NAD	C2N-N1N	2.26	1.37	1.35
3	A	402	NAD	C8A-N7A	-2.23	1.30	1.34
3	B	402	NAD	C2N-N1N	2.04	1.37	1.35

All (21) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
4	B	403	A1I3Z	C3-C2-S1	-3.94	106.21	113.79
4	A	403	A1I3Z	C3-C2-S1	-3.32	107.41	113.79
3	A	402	NAD	C5A-C6A-N6A	3.13	125.11	120.35
4	A	403	A1I3Z	C1-S1-C2	2.78	119.96	99.77
4	B	403	A1I3Z	C24-C9-C10	2.75	126.27	120.14
2	B	401	FAD	C5A-C6A-N6A	2.63	124.35	120.35
4	B	403	A1I3Z	C8-C9-C10	-2.51	113.93	120.29
3	A	402	NAD	C6N-N1N-C2N	-2.49	119.70	121.97
4	B	403	A1I3Z	C1-S1-C2	2.40	117.22	99.77
4	A	403	A1I3Z	C12-C11-N2	2.39	114.46	110.82
4	A	403	A1I3Z	C6-C5-N1	-2.35	117.10	119.15
4	A	403	A1I3Z	C24-C9-C10	2.33	125.33	120.14
2	A	401	FAD	C5A-C6A-N6A	2.31	123.86	120.35
4	A	403	A1I3Z	C24-C5-N1	2.21	121.89	119.07
2	B	401	FAD	O2A-PA-O1A	2.20	123.10	112.24
2	A	401	FAD	C4-N3-C2	-2.18	121.61	125.64
4	A	403	A1I3Z	C13-C14-N3	-2.14	121.01	125.08
3	B	402	NAD	C6N-N1N-C2N	-2.05	120.11	121.97

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Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
4	A	403	A1I3Z	C8-C9-C10	-2.04	115.12	120.29
2	B	401	FAD	C4-N3-C2	-2.03	121.89	125.64
3	B	402	NAD	O2A-PA-O1A	2.02	122.23	112.24

There are no chirality outliers.

All (27) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	B	401	FAD	PA-O3P-P-O5'
4	B	403	A1I3Z	C3-C2-S1-C1
6	A	405	PEG	O2-C3-C4-O4
6	A	405	PEG	C4-C3-O2-C2
6	A	405	PEG	O1-C1-C2-O2
4	A	403	A1I3Z	C3-C2-S1-C1
2	A	401	FAD	PA-O3P-P-O5'
2	B	401	FAD	P-O3P-PA-O1A
2	A	401	FAD	O2'-C2'-C3'-C4'
2	A	401	FAD	O2'-C2'-C3'-O3'
4	B	403	A1I3Z	N3-C15-C16-C17
2	B	401	FAD	P-O3P-PA-O2A
4	A	403	A1I3Z	C24-C5-N1-C4
2	B	401	FAD	O4B-C4B-C5B-O5B
2	B	401	FAD	O2'-C2'-C3'-O3'
2	A	401	FAD	P-O3P-PA-O1A
4	A	403	A1I3Z	C6-C5-N1-C4
2	A	401	FAD	O3'-C3'-C4'-C5'
4	A	403	A1I3Z	C6-C5-N1-N6
4	A	403	A1I3Z	C24-C5-N1-N6
2	B	401	FAD	O2'-C2'-C3'-C4'
3	A	402	NAD	O4B-C4B-C5B-O5B
3	B	402	NAD	O4B-C4B-C5B-O5B
2	A	401	FAD	P-O3P-PA-O2A
2	A	401	FAD	O4B-C4B-C5B-O5B
2	B	401	FAD	C1'-C2'-C3'-O3'
4	B	403	A1I3Z	N3-C15-C16-C21

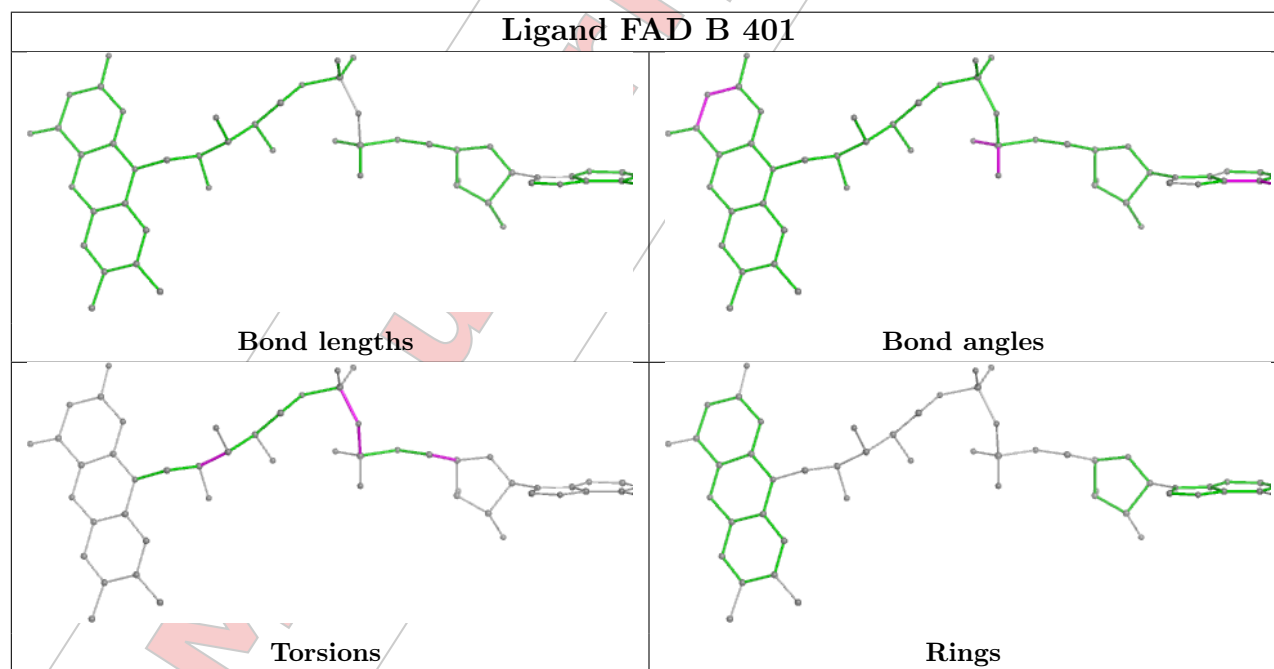
All (2) ring outliers are listed below:

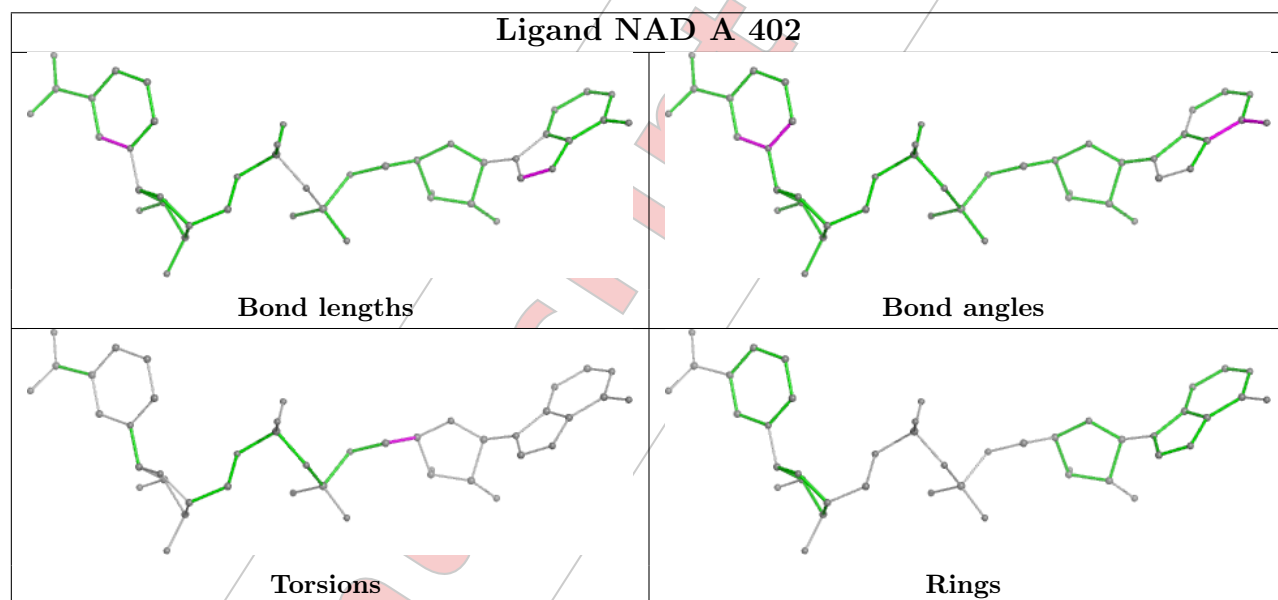
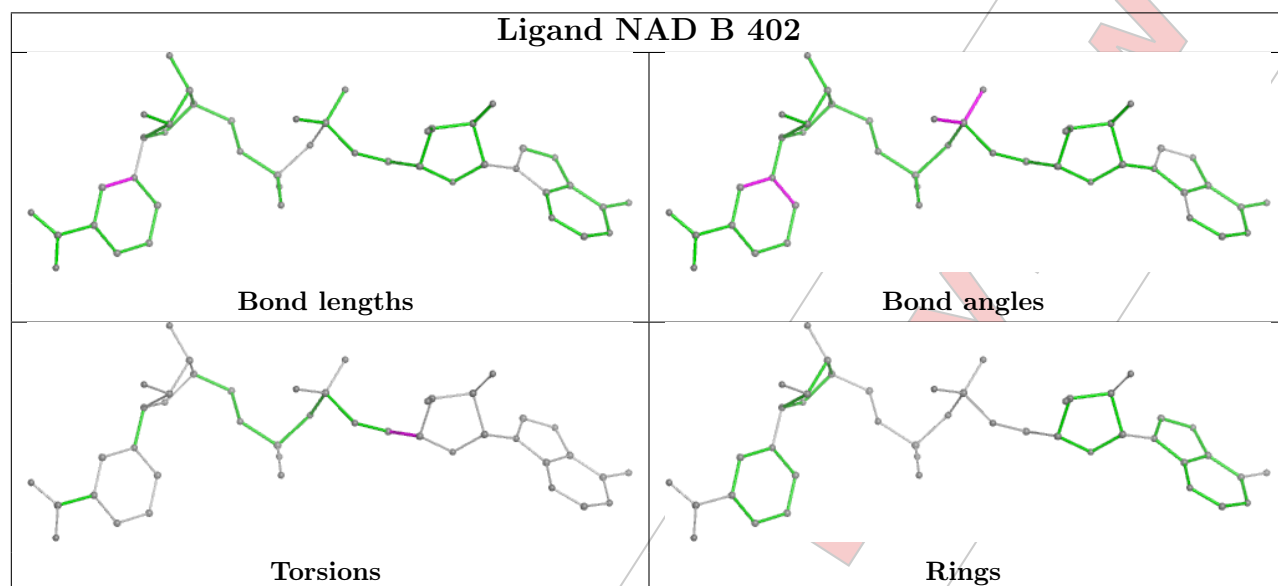
Mol	Chain	Res	Type	Atoms
4	A	403	A1I3Z	C11-C12-C13-C22-C23-N2
4	B	403	A1I3Z	C11-C12-C13-C22-C23-N2

4 monomers are involved in 10 short contacts:

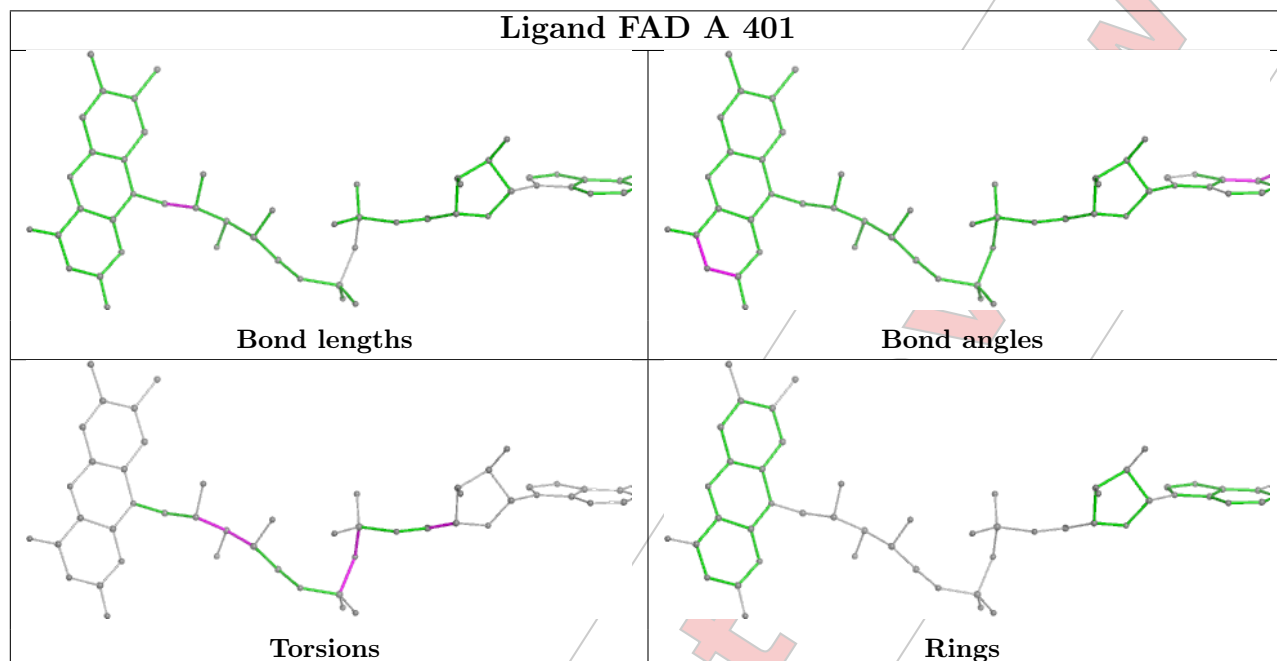
Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	401	FAD	5	0
4	A	403	A1I3Z	1	0
6	A	405	PEG	2	0
4	B	403	A1I3Z	2	0

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.

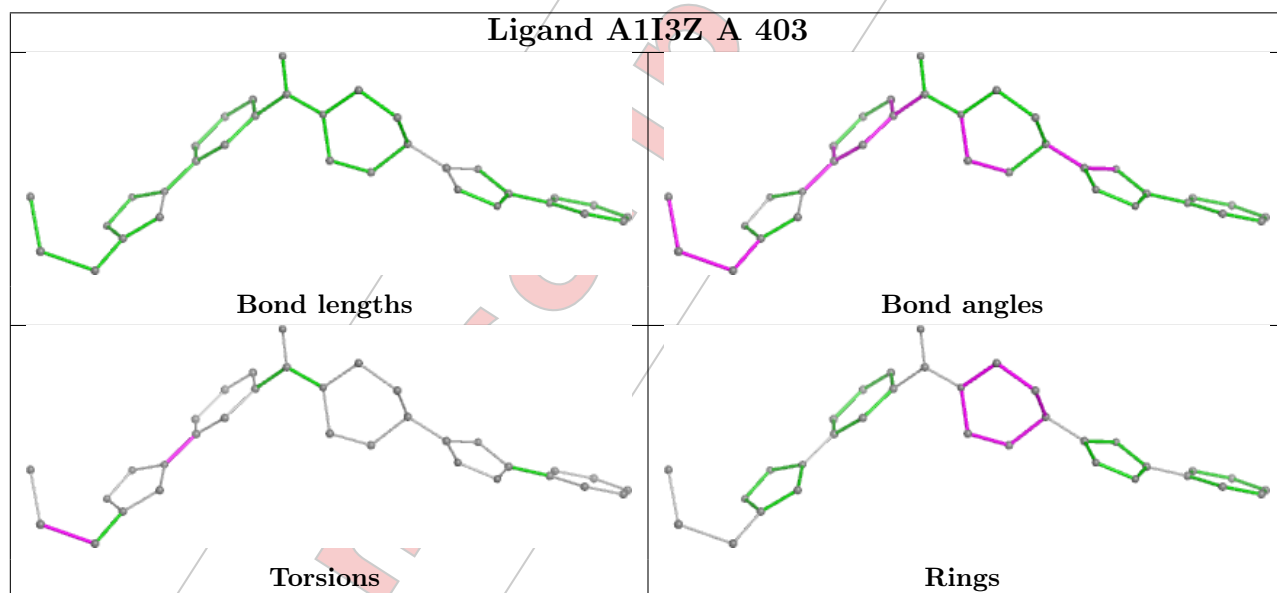


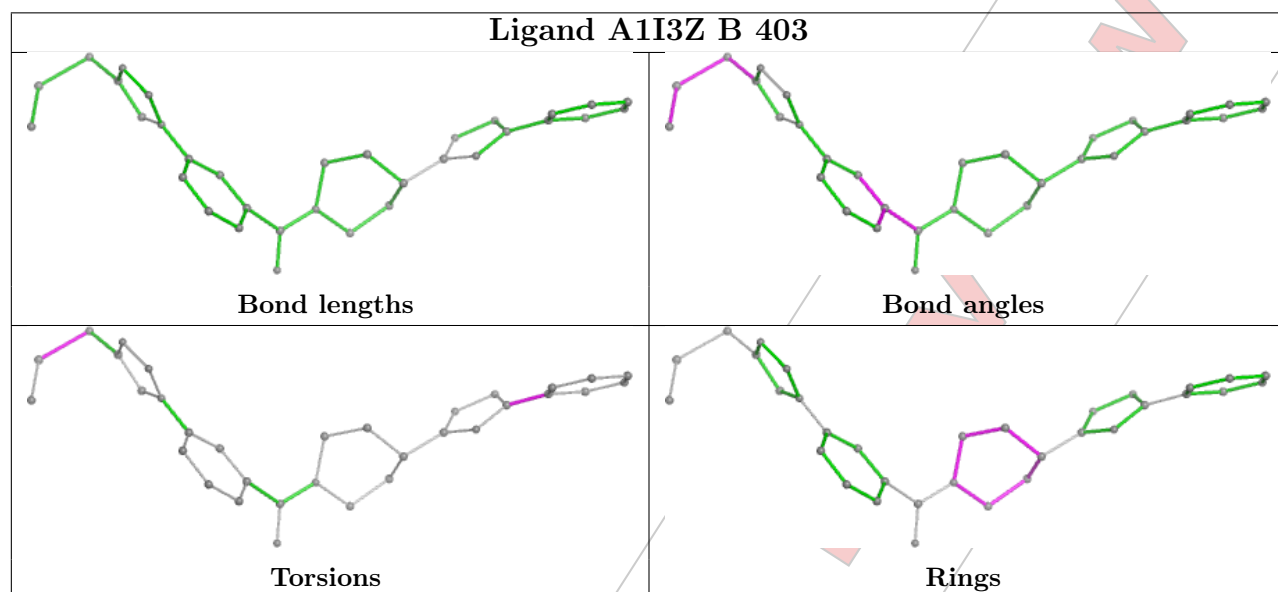


Ligand FAD A 401



Ligand A1I3Z A 403





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, 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	364/368 (98%)	-0.32	2 (0%) 87 91	8, 19, 34, 56	2 (0%)
1	B	364/368 (98%)	0.25	12 (3%) 49 57	10, 28, 49, 79	3 (0%)
All	All	728/736 (98%)	-0.03	14 (1%) 66 72	8, 23, 45, 79	5 (0%)

All (14) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	5	SER	3.2
1	B	254	LEU	3.2
1	B	255	ALA	3.1
1	B	251	GLY	3.0
1	B	5	SER	2.6
1	B	250	PHE	2.5
1	B	258	GLY	2.4
1	B	224	LYS	2.3
1	B	283	VAL	2.2
1	B	82	GLU	2.2
1	B	257	ASN	2.1
1	B	83	LYS	2.1
1	B	344	VAL	2.0
1	A	207	GLN	2.0

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 ⓘ

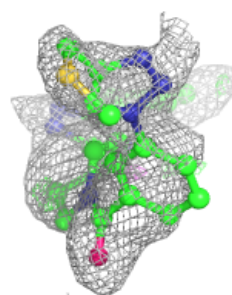
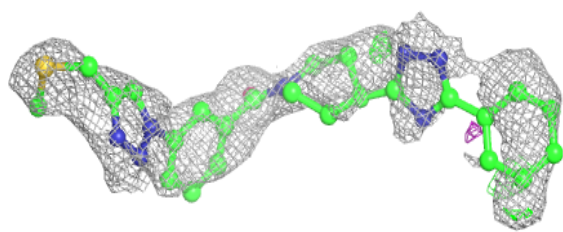
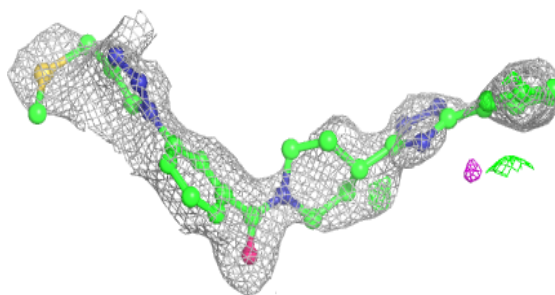
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95th percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists 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	A1I3Z	B	403	33/33	0.80	0.17	44,67,87,88	0
4	A1I3Z	A	403	33/33	0.86	0.15	27,49,59,63	0
5	IMD	A	404	5/5	0.87	0.15	40,42,44,44	0
7	CA	A	406	1/1	0.91	0.09	48,48,48,48	0
6	PEG	A	405	7/7	0.92	0.10	24,31,36,37	0
7	CA	B	404	1/1	0.95	0.19	61,61,61,61	0
7	CA	B	407	1/1	0.95	0.07	35,35,35,35	0
8	CL	A	407	1/1	0.96	0.10	35,35,35,35	0
2	FAD	B	401	53/53	0.97	0.06	15,20,26,29	0
7	CA	B	405	1/1	0.97	0.24	46,46,46,46	0
9	MG	A	408	1/1	0.97	0.18	33,33,33,33	0
7	CA	B	406	1/1	0.98	0.20	50,50,50,50	0
3	NAD	B	402	44/44	0.98	0.05	19,23,28,31	0
2	FAD	A	401	53/53	0.99	0.04	11,14,17,19	0
3	NAD	A	402	44/44	0.99	0.04	12,16,19,21	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.

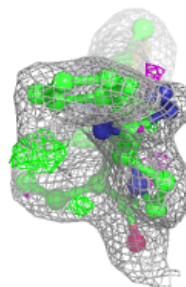
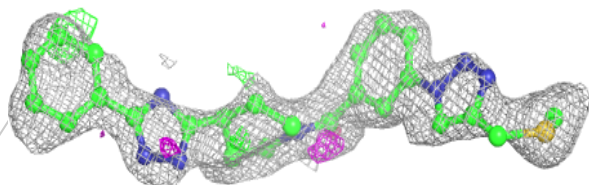
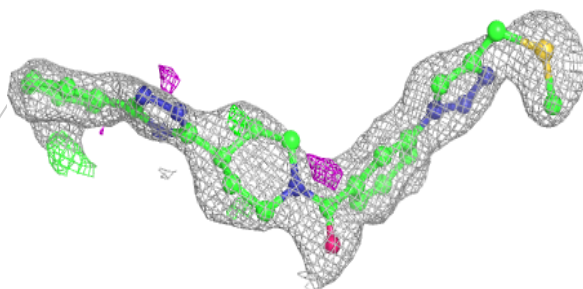
Electron density around A1I3Z B 403:

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



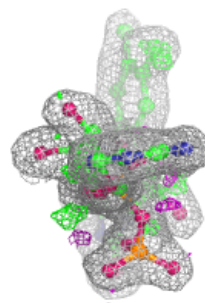
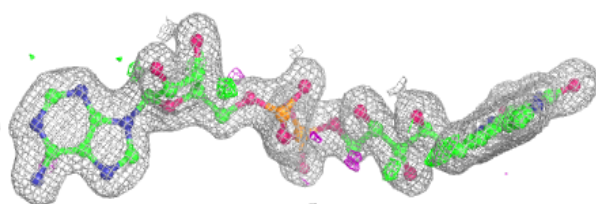
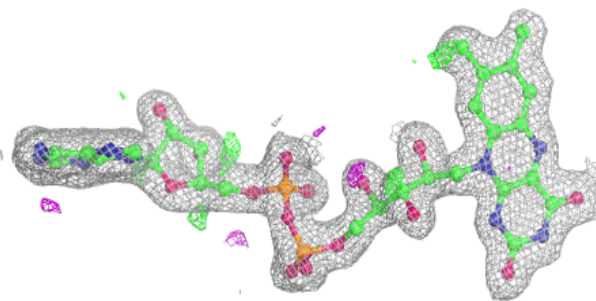
Electron density around A1I3Z A 403:

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



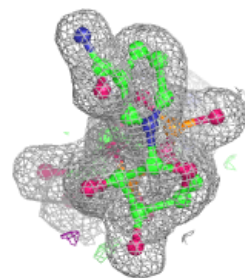
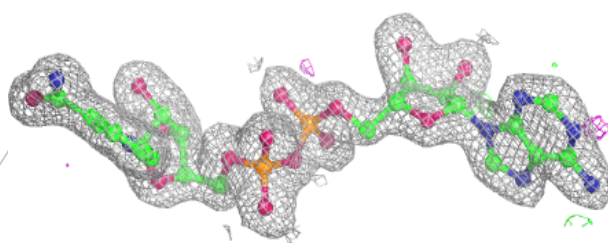
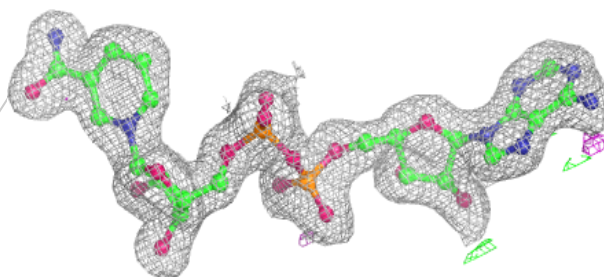
Electron density around FAD B 401:

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



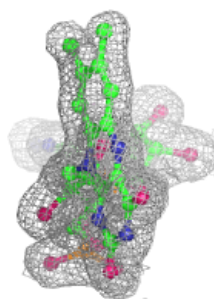
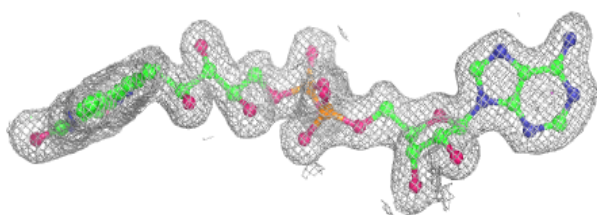
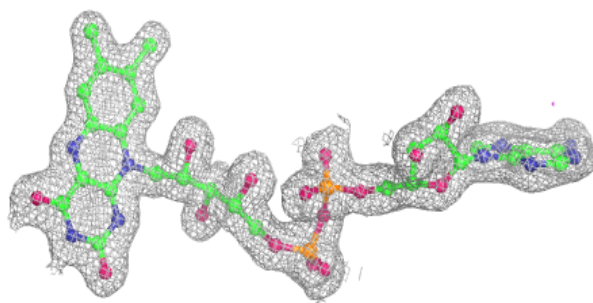
Electron density around NAD B 402:

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



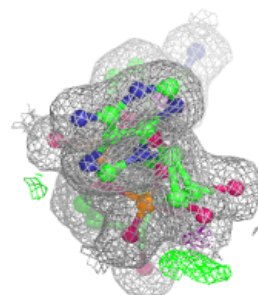
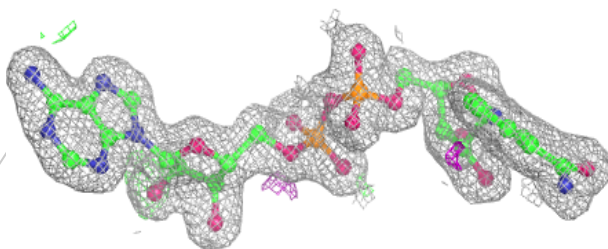
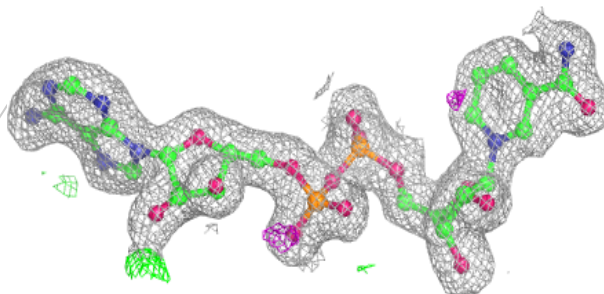
Electron density around FAD A 401:

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



Electron density around NAD A 402:

$2mF_o-DF_c$ (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.

For Manuscript Review



Full wwPDB X-ray Structure Validation Report ⓘ

Feb 19, 2025 – 11:06 am GMT

PDB ID : 9IFY
Title : FSP1 (tetrapod ancestor) bound to FAD and NAD⁺ and coenzyme Q1
Deposited on : 2025-02-18
Resolution : 1.70 Å (reported)

This wwPDB validation report is for manuscript review

This is a Full wwPDB X-ray Structure Validation Report.

This report is produced by the wwPDB biocuration pipeline after annotation of the structure.

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 ⓘ symbol.

The types of validation reports are described at

<https://www.wwpdb.org/validation/2017/FAQs#types>.

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

MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (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	:	9.0.003 (Gargrove)
Density-Fitness	:	1.0.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)

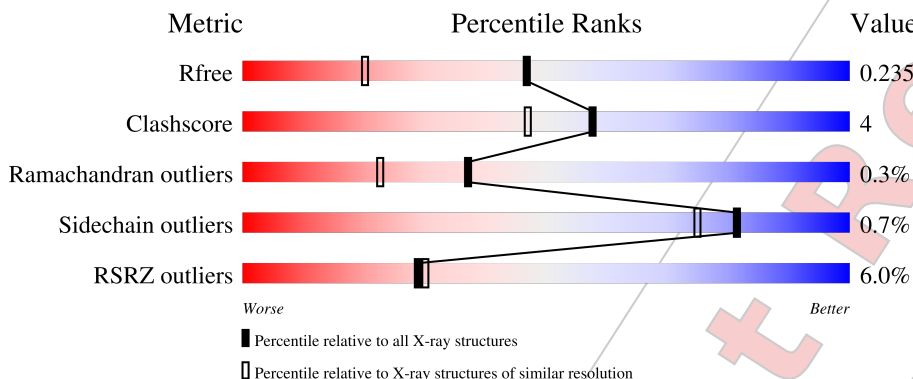
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 1.70 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	5161 (1.70-1.70)
Clashscore	180529	5671 (1.70-1.70)
Ramachandran outliers	177936	5594 (1.70-1.70)
Sidechain outliers	177891	5594 (1.70-1.70)
RSRZ outliers	164620	5159 (1.70-1.70)

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 $\leq 5\%$. 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	368	94% ..
1	B	368	11% 89% 10% .

The following table lists non-polymeric compounds, carbohydrate monomers and non-standard residues in protein, DNA, RNA chains that are outliers for geometric or electron-density-fit criteria.

Validation Pipeline (wwPDB-VP) : 2.41

ria:

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
4	UQ1	A	403	-	-	X	-
4	UQ1	B	404	-	-	X	-

2 Entry composition [i](#)

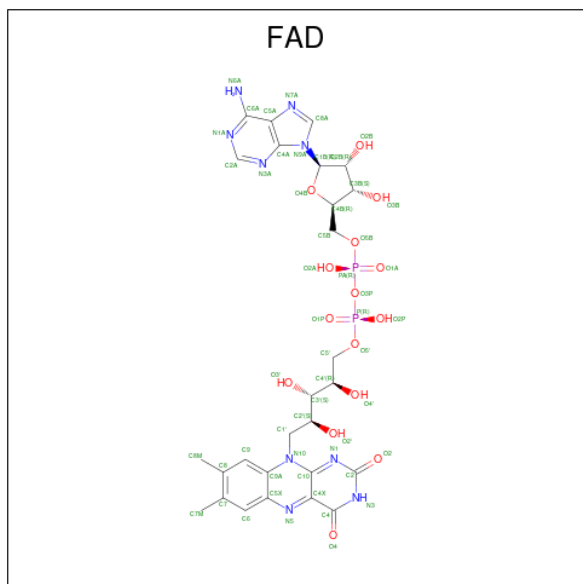
There are 7 unique types of molecules in this entry. The entry contains 6197 atoms, of which 0 are hydrogens and 0 are deuteriums.

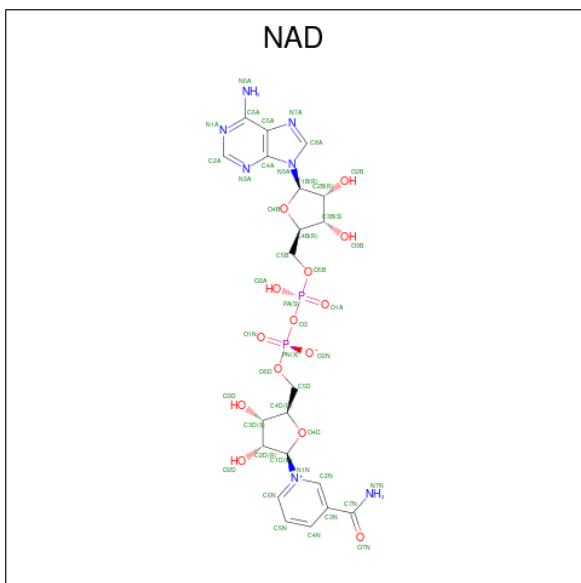
In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

- Molecule 1 is a protein called FSP1 (tetrapod ancestor).

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	365	Total	C	N	O	S	0	3	0
			2792	1771	479	528	14			
1	B	365	Total	C	N	O	S	0	4	0
			2799	1774	479	532	14			

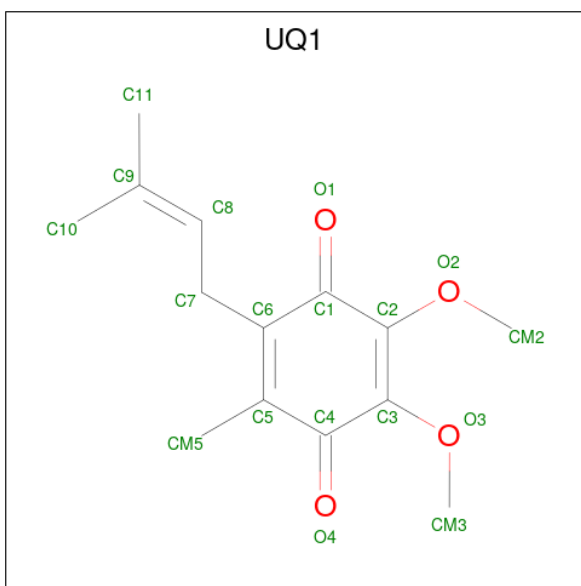
- Molecule 2 is FLAVIN-ADENINE DINUCLEOTIDE (three-letter code: FAD) (formula: $C_{27}H_{33}N_9O_{15}P_2$).





Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
3	A	1	Total	C	N	O	P	0	0
			44	21	7	14	2		
3	B	1	Total	C	N	O	P	0	0
			44	21	7	14	2		

- Molecule 4 is UBIQUINONE-1 (three-letter code: UQ1) (formula: $C_{14}H_{18}O_4$) (labeled as "Ligand of Interest" by depositor).



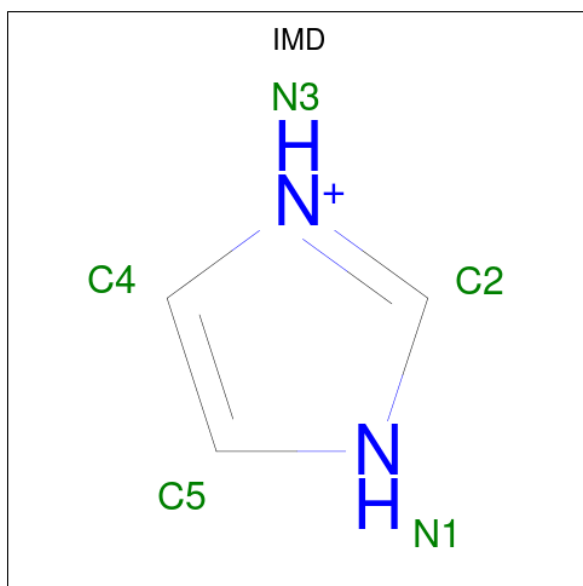
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
4	A	1	Total	C	O	0	0
			18	14	4		

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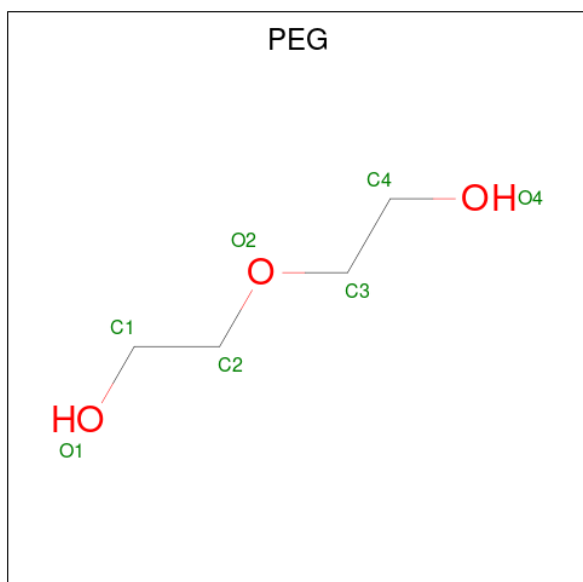
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
4	B	1	Total	C	O	0	0
			18	14	4		

- Molecule 5 is IMIDAZOLE (three-letter code: IMD) (formula: $C_3H_5N_2$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
5	A	1	Total	C	N	0	0
			5	3	2		

- Molecule 6 is DI(HYDROXYETHYL)ETHER (three-letter code: PEG) (formula: $C_4H_{10}O_3$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
6	B	1	Total	C	O	0	0
			7	4	3		

- Molecule 7 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
7	A	254	Total	O	0	0
			254	254		
7	B	110	Total	O	0	0
			110	110		

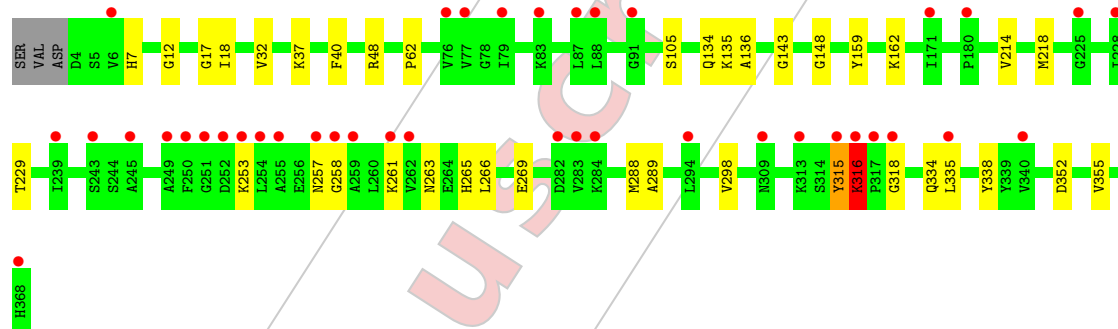
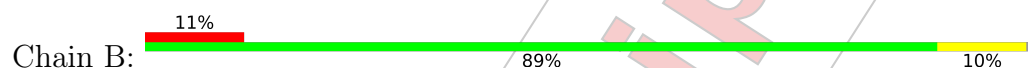
3 Residue-property plots

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: FSP1 (tetrapod ancestor)



- Molecule 1: FSP1 (tetrapod ancestor)



4 Data and refinement statistics i

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α , β , γ	43.51Å 79.15Å 112.07Å 90.00° 98.79° 90.00°	Depositor
Resolution (Å)	37.81 – 1.70 37.81 – 1.70	Depositor EDS
% Data completeness (in resolution range)	95.7 (37.81-1.70) 95.7 (37.81-1.70)	Depositor EDS
R_{merge}	0.04	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.46 (at 1.70Å)	Xtriage
Refinement program	REFMAC 5.8.0405	Depositor
R, R_{free}	0.188 , 0.226 0.202 , 0.235	Depositor DCC
R_{free} test set	4057 reflections (4.91%)	wwPDB-VP
Wilson B-factor (Å ²)	24.5	Xtriage
Anisotropy	0.008	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.36 , 38.5	EDS
L-test for twinning ²	$\langle L \rangle = 0.49$, $\langle L^2 \rangle = 0.32$	Xtriage
Estimated twinning fraction	0.018 for h,-k,-h-l	Xtriage
F_o, F_c correlation	0.96	EDS
Total number of atoms	6197	wwPDB-VP
Average B, all atoms (Å ²)	31.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 4.95% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical 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: UQ1, FAD, PEG, IMD, NAD

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	0.45	0/2840	0.75	1/3834 (0.0%)
1	B	0.38	0/2847	0.72	1/3843 (0.0%)
All	All	0.42	0/5687	0.73	2/7677 (0.0%)

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	B	0	1

There are no bond length outliers.

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	326	MET	CG-SD-CE	-9.00	85.79	100.20
1	B	318	GLY	N-CA-C	5.02	125.66	113.10

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	B	48	ARG	Sidechain

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	2792	0	2821	11	0
1	B	2799	0	2826	27	0
2	A	53	0	31	1	0
2	B	53	0	31	1	0
3	A	44	0	26	1	0
3	B	44	0	26	0	0
4	A	18	0	18	8	0
4	B	18	0	18	9	0
5	A	5	0	5	0	0
6	B	7	0	10	1	0
7	A	254	0	0	0	0
7	B	110	0	0	0	0
All	All	6197	0	5812	51	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.

All (51) 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)
4:A:403:UQ1:HM32	4:A:403:UQ1:CM2	1.78	1.13
4:B:404:UQ1:HM33	4:B:404:UQ1:CM2	1.86	1.05
4:B:404:UQ1:HM23	4:B:404:UQ1:CM3	1.88	1.03
4:A:403:UQ1:HM32	4:A:403:UQ1:HM23	1.06	1.02
4:B:404:UQ1:HM33	4:B:404:UQ1:HM23	0.95	0.94
4:B:404:UQ1:HM51	4:B:404:UQ1:C8	1.99	0.93
4:A:403:UQ1:HM23	4:A:403:UQ1:CM3	1.99	0.93
4:A:403:UQ1:CM2	4:A:403:UQ1:CM3	2.53	0.85
1:B:289:ALA:HB3	4:B:404:UQ1:HM31	1.70	0.73
4:A:403:UQ1:HM51	4:A:403:UQ1:H103	1.71	0.71
1:B:134:GLN:HG3	1:B:159:TYR:OH	1.94	0.68
1:A:7:HIS:NE2	1:A:32:VAL:HG23	2.11	0.65
4:B:404:UQ1:HM51	4:B:404:UQ1:H8	1.77	0.65
1:A:18:ILE:HD12	1:A:40:PHE:CD1	2.34	0.63
1:B:263:ASN:OD1	1:B:265:HIS:N	2.32	0.58
1:B:18:ILE:CD1	1:B:40:PHE:CD1	2.87	0.57
1:B:266:LEU:HD12	1:B:298:VAL:CG1	2.36	0.55
4:A:403:UQ1:CM3	4:A:403:UQ1:HM22	2.36	0.55
1:B:7:HIS:NE2	1:B:32:VAL:HG23	2.24	0.53

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:B:315:TYR:O	1:B:316:LYS:HB2	2.08	0.53
1:B:257:ASN:OD1	1:B:258:GLY:N	2.42	0.52
1:A:18:ILE:CD1	1:A:40:PHE:CD1	2.94	0.50
1:B:134:GLN:CG	1:B:159:TYR:OH	2.59	0.50
1:A:348:ALA:HB1	4:A:403:UQ1:H112	1.94	0.50
1:B:135:LYS:HB2	1:B:214:VAL:HG21	1.94	0.50
1:B:266:LEU:HD12	1:B:298:VAL:HG11	1.95	0.48
1:B:136:ALA:O	1:B:162:LYS:NZ	2.30	0.47
1:B:159:TYR:HB3	1:B:162:LYS:HG3	1.98	0.46
1:B:253:LYS:HE2	1:B:269:GLU:O	2.15	0.45
1:B:334:GLN:HA	1:B:338:TYR:O	2.15	0.45
1:B:288:MET:HA	2:B:401:FAD:H1'2	1.98	0.45
1:B:62:PRO:HB3	6:B:403:PEG:H32	1.99	0.44
4:A:403:UQ1:HM51	4:A:403:UQ1:C10	2.45	0.43
4:B:404:UQ1:C8	4:B:404:UQ1:CM5	2.81	0.43
1:A:287:LYS:O	3:A:402:NAD:H1D	2.18	0.43
1:B:335:LEU:HD13	4:B:404:UQ1:H102	2.00	0.43
1:B:37[B]:LYS:NZ	1:B:37[B]:LYS:HB2	2.34	0.42
1:A:326:MET:HG2	1:A:331:GLY:HA2	2.02	0.42
1:B:18:ILE:HD12	1:B:40:PHE:CD1	2.54	0.42
1:A:14:GLY:HA3	2:A:401:FAD:O5B	2.20	0.41
1:A:106:ASP:HB2	1:A:242:ASN:OD1	2.21	0.41
1:B:18:ILE:HD11	1:B:40:PHE:HB2	2.02	0.41
1:B:218:MET:O	1:B:229:THR:HA	2.20	0.41
1:B:253:LYS:O	1:B:261:LYS:HG3	2.20	0.41
1:B:143:GLY:O	1:B:148:GLY:HA3	2.20	0.41
1:B:352:ASP:O	1:B:355:VAL:HG22	2.21	0.41
1:A:11:VAL:HG13	1:A:76:VAL:HG21	2.03	0.41
1:A:50:SER:HB3	1:A:129:MET:HG2	2.01	0.41
1:B:289:ALA:CB	4:B:404:UQ1:HM31	2.46	0.41
1:B:12:GLY:O	1:B:17:GLY:HA3	2.20	0.40
1:A:45:ALA:HB1	1:A:55:PHE:HE2	1.86	0.40

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	366/368 (100%)	359 (98%)	7 (2%)	0	100	100
1	B	367/368 (100%)	355 (97%)	10 (3%)	2 (0%)	25	12
All	All	733/736 (100%)	714 (97%)	17 (2%)	2 (0%)	37	23

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	B	316	LYS
1	B	315	TYR

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	301/302 (100%)	299 (99%)	2 (1%)	81	75
1	B	303/302 (100%)	301 (99%)	2 (1%)	81	75
All	All	604/604 (100%)	600 (99%)	4 (1%)	81	75

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

Mol	Chain	Res	Type
1	A	129	MET
1	A	352	ASP
1	B	105	SER
1	B	316	LYS

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

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 oligosaccharides in this entry.

5.6 Ligand geometry ⓘ

8 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	FAD	A	401	-	53,58,58	0.88	2 (3%)	68,89,89	0.94	4 (5%)
2	FAD	B	401	-	53,58,58	0.72	0	68,89,89	0.90	1 (1%)
3	NAD	A	402	-	42,48,48	0.74	2 (4%)	50,73,73	0.84	1 (2%)
3	NAD	B	402	-	42,48,48	0.71	1 (2%)	50,73,73	0.80	1 (2%)
4	UQ1	B	404	-	18,18,18	0.92	1 (5%)	22,25,25	1.01	0
5	IMD	A	404	-	3,5,5	0.43	0	4,5,5	0.71	0
4	UQ1	A	403	-	18,18,18	0.77	0	22,25,25	1.15	2 (9%)
6	PEG	B	403	-	6,6,6	0.73	0	5,5,5	0.50	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
2	FAD	A	401	-	-	2/30/50/50	0/6/6/6

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Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
2	FAD	B	401	-	-	8/30/50/50	0/6/6/6
3	NAD	A	402	-	-	1/26/62/62	0/5/5/5
3	NAD	B	402	-	-	1/26/62/62	0/5/5/5
4	UQ1	B	404	-	-	3/9/33/33	0/1/1/1
5	IMD	A	404	-	-	-	0/1/1/1
4	UQ1	A	403	-	-	5/9/33/33	0/1/1/1
6	PEG	B	403	-	-	1/4/4/4	-

All (6) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	401	FAD	C1'-C2'	-3.72	1.47	1.52
3	B	402	NAD	C2N-N1N	2.49	1.38	1.35
3	A	402	NAD	C8A-N7A	-2.30	1.30	1.34
4	B	404	UQ1	C6-C5	2.22	1.39	1.35
3	A	402	NAD	C2N-N1N	2.17	1.37	1.35
2	A	401	FAD	C6-C7	-2.17	1.36	1.39

All (9) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	B	401	FAD	O2A-PA-O1A	2.80	126.08	112.24
3	A	402	NAD	C6N-N1N-C2N	-2.32	119.86	121.97
4	A	403	UQ1	O2-C2-C3	2.28	132.25	123.64
3	B	402	NAD	C6N-N1N-C2N	-2.27	119.90	121.97
4	A	403	UQ1	O2-C2-C1	-2.25	108.94	116.56
2	A	401	FAD	O2A-PA-O1A	2.23	123.25	112.24
2	A	401	FAD	C4-N3-C2	-2.17	121.63	125.64
2	A	401	FAD	O2P-P-O1P	2.11	122.69	112.24
2	A	401	FAD	O3'-C3'-C2'	2.08	113.84	108.81

There are no chirality outliers.

All (21) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	B	401	FAD	C1'-C2'-C3'-O3'
2	B	401	FAD	C1'-C2'-C3'-C4'
4	B	404	UQ1	C1-C6-C7-C8
4	B	404	UQ1	C5-C6-C7-C8
2	B	401	FAD	O2'-C2'-C3'-C4'

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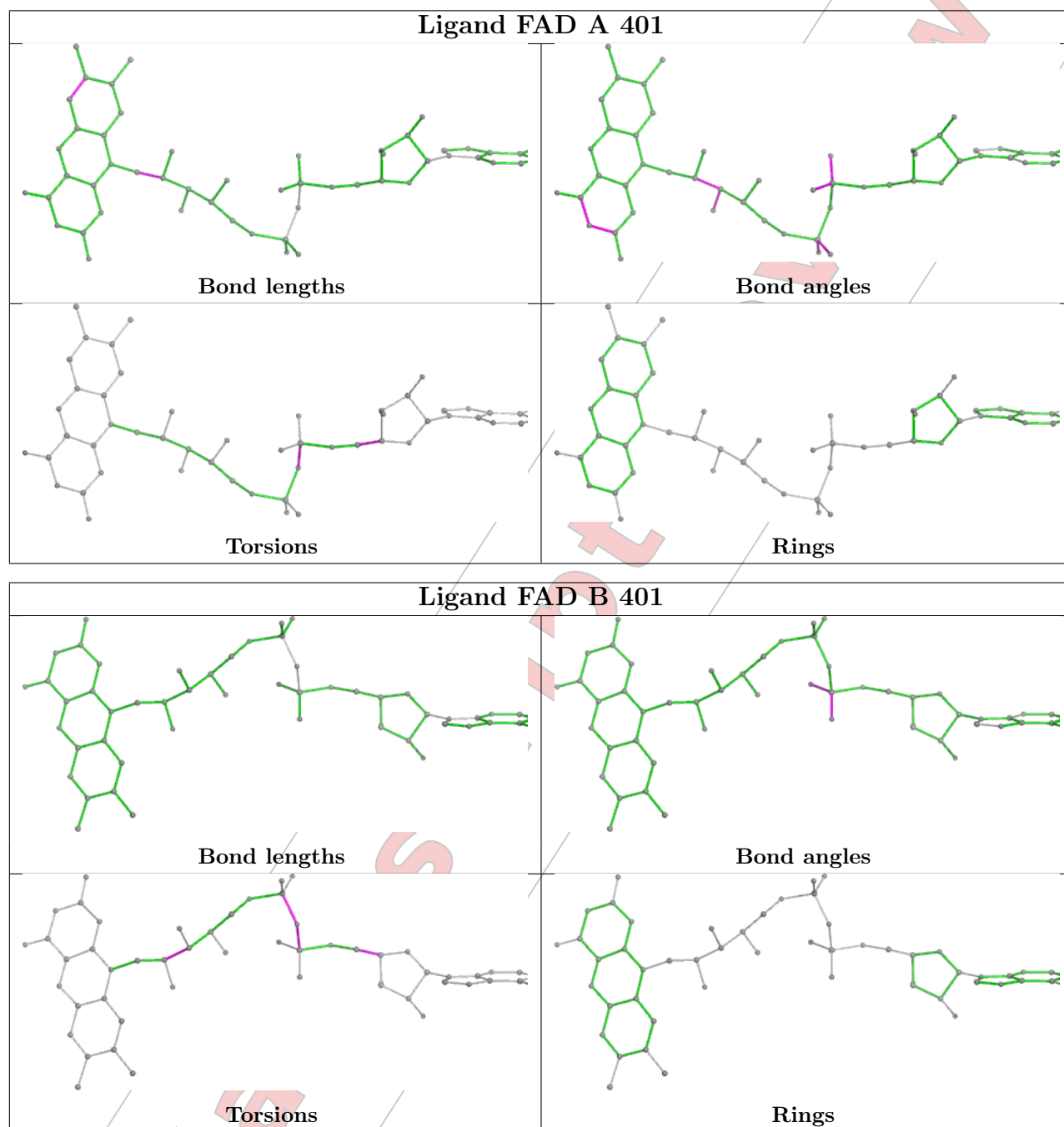
Mol	Chain	Res	Type	Atoms
2	B	401	FAD	O2'-C2'-C3'-O3'
6	B	403	PEG	O2-C3-C4-O4
4	A	403	UQ1	C7-C8-C9-C11
2	B	401	FAD	PA-O3P-P-O5'
2	B	401	FAD	O4B-C4B-C5B-O5B
4	B	404	UQ1	C4-C3-O3-CM3
2	B	401	FAD	P-O3P-PA-O1A
4	A	403	UQ1	C4-C3-O3-CM3
3	B	402	NAD	O4B-C4B-C5B-O5B
2	A	401	FAD	P-O3P-PA-O2A
2	B	401	FAD	P-O3P-PA-O2A
2	A	401	FAD	O4B-C4B-C5B-O5B
3	A	402	NAD	O4B-C4B-C5B-O5B
4	A	403	UQ1	C1-C2-O2-CM2
4	A	403	UQ1	C7-C8-C9-C10
4	A	403	UQ1	C3-C2-O2-CM2

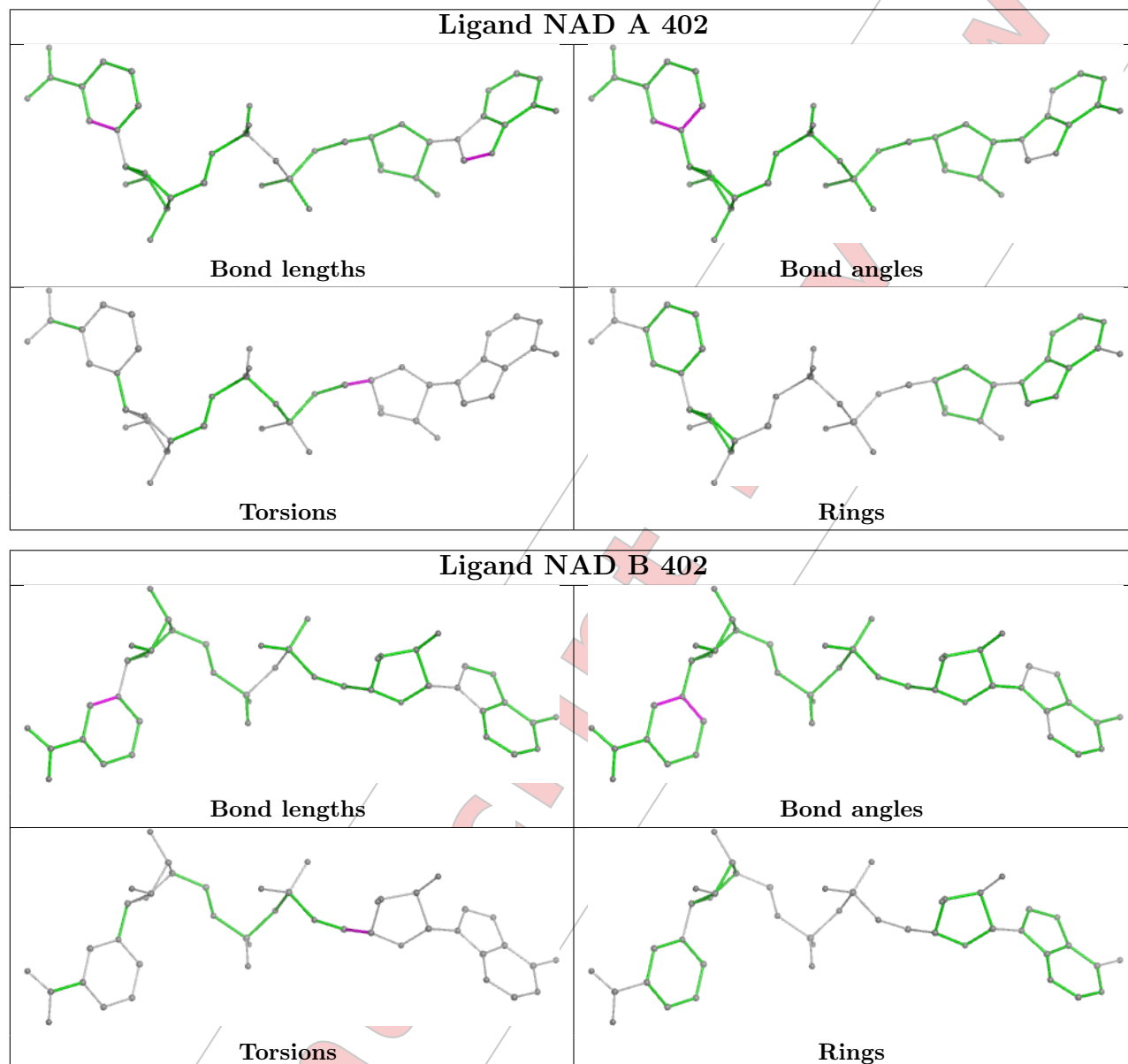
There are no ring outliers.

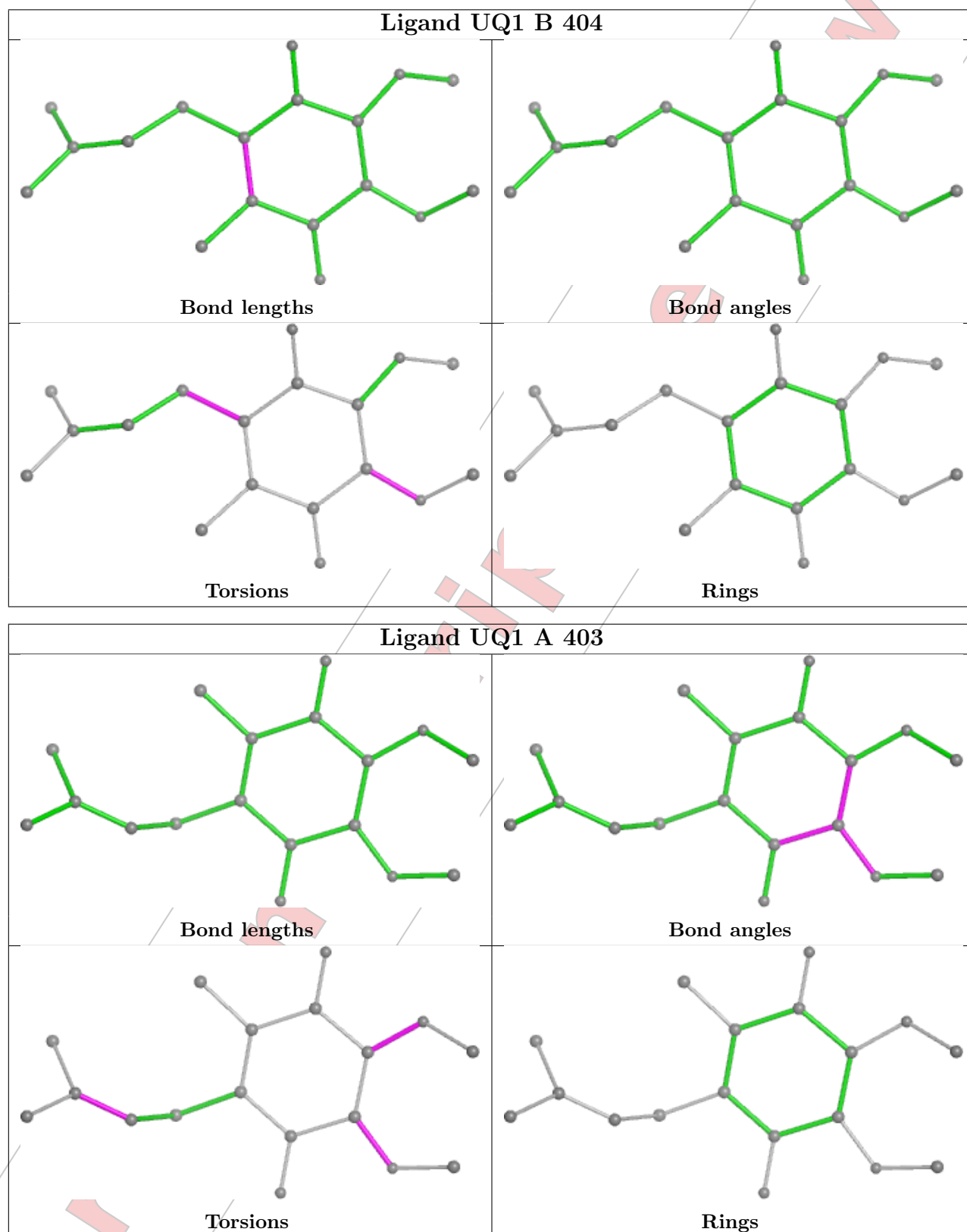
6 monomers are involved in 21 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	401	FAD	1	0
2	B	401	FAD	1	0
3	A	402	NAD	1	0
4	B	404	UQ1	9	0
4	A	403	UQ1	8	0
6	B	403	PEG	1	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 ⓘ

There are no such residues in this entry.

5.8 Polymer linkage issues ⓘ

There are no chain breaks in this entry.

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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, 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	365/368 (99%)	-0.10	4 (1%) 77 80	9, 22, 41, 67	3 (0%)
1	B	365/368 (99%)	0.79	40 (10%) 12 11	14, 34, 60, 88	4 (1%)
All	All	730/736 (99%)	0.34	44 (6%) 29 30	9, 27, 55, 88	7 (0%)

All (44) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	B	255	ALA	5.6
1	B	254	LEU	4.9
1	B	315	TYR	3.9
1	B	317	PRO	3.7
1	A	4	ASP	3.5
1	B	283	VAL	3.5
1	B	251	GLY	3.3
1	B	259	ALA	3.3
1	B	77	VAL	3.3
1	B	180	PRO	3.3
1	A	368	HIS	3.0
1	A	5	SER	3.0
1	B	257	ASN	2.9
1	B	245	ALA	2.9
1	B	249	ALA	2.9
1	A	7	HIS	2.9
1	B	83	LYS	2.8
1	B	316	LYS	2.8
1	B	294	LEU	2.8
1	B	262	VAL	2.8
1	B	250	PHE	2.8
1	B	6	VAL	2.7
1	B	87	LEU	2.6
1	B	309	ASN	2.5

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Mol	Chain	Res	Type	RSRZ
1	B	258	GLY	2.5
1	B	171	ILE	2.5
1	B	335	LEU	2.4
1	B	318	GLY	2.4
1	B	252	ASP	2.4
1	B	228	ILE	2.4
1	B	284	LYS	2.4
1	B	313	LYS	2.4
1	B	261	LYS	2.2
1	B	79	ILE	2.2
1	B	253	LYS	2.2
1	B	282	ASP	2.2
1	B	340	VAL	2.2
1	B	368	HIS	2.2
1	B	239	ILE	2.2
1	B	91	GLY	2.2
1	B	225	GLY	2.2
1	B	243	SER	2.1
1	B	76	VAL	2.1
1	B	88	LEU	2.1

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, 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($\text{\AA}^2$)	Q<0.9
4	UQ1	B	404	18/18	0.73	0.24	65,73,82,85	0
4	UQ1	A	403	18/18	0.80	0.18	36,48,64,69	0
6	PEG	B	403	7/7	0.84	0.14	30,33,40,40	0

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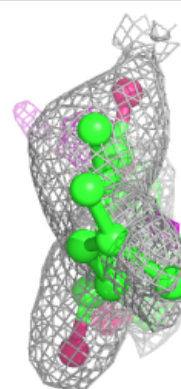
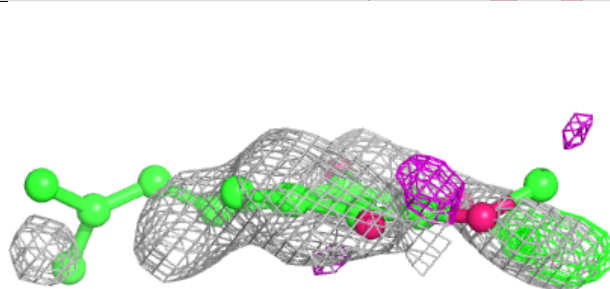
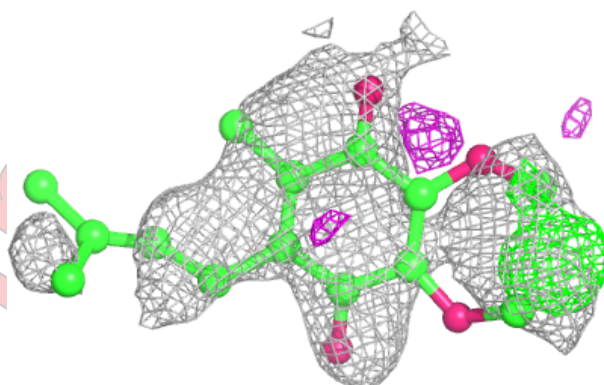
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Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors($\text{\AA}^2$)	Q<0.9
5	IMD	A	404	5/5	0.86	0.18	39,39,41,47	0
3	NAD	B	402	44/44	0.94	0.09	29,35,39,42	0
2	FAD	B	401	53/53	0.96	0.08	22,28,38,40	0
3	NAD	A	402	44/44	0.98	0.04	15,18,22,25	0
2	FAD	A	401	53/53	0.98	0.05	14,17,21,26	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.

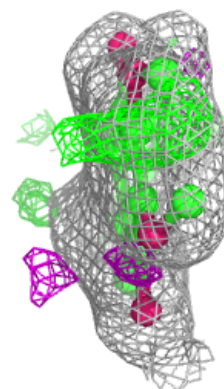
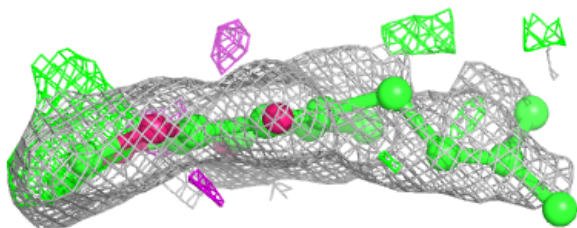
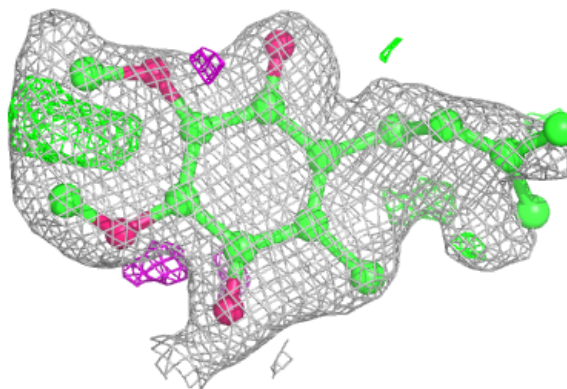
Electron density around UQ1 B 404:

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



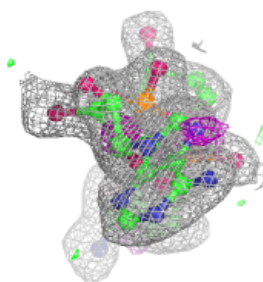
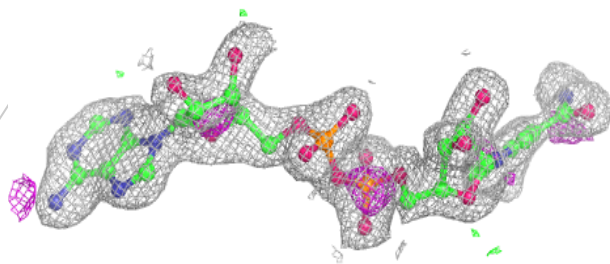
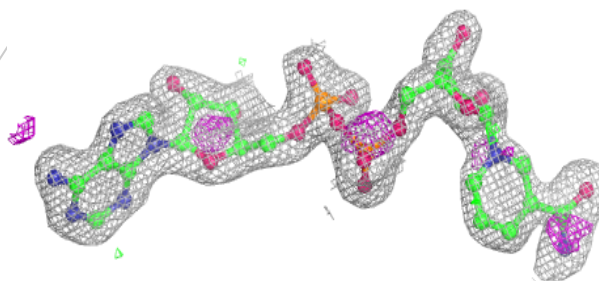
Electron density around UQ1 A 403:

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



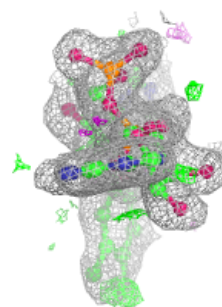
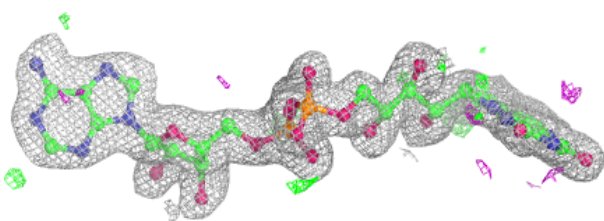
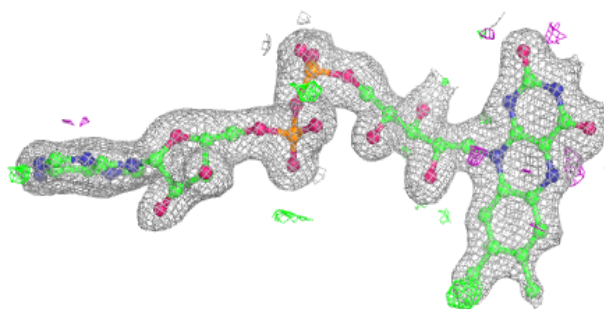
Electron density around NAD B 402:

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



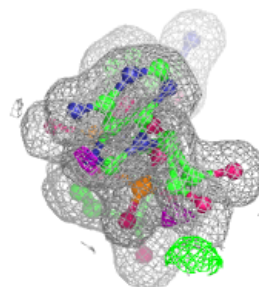
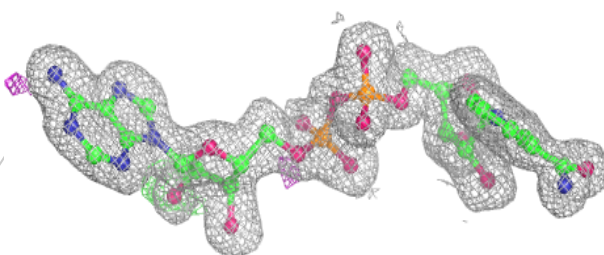
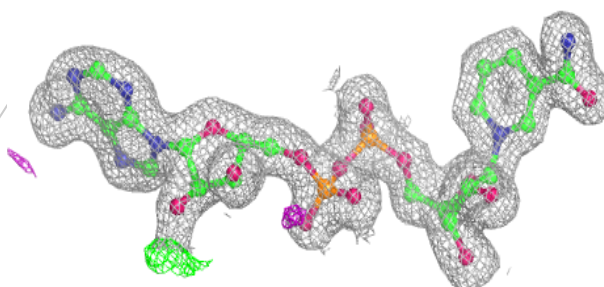
Electron density around FAD B 401:

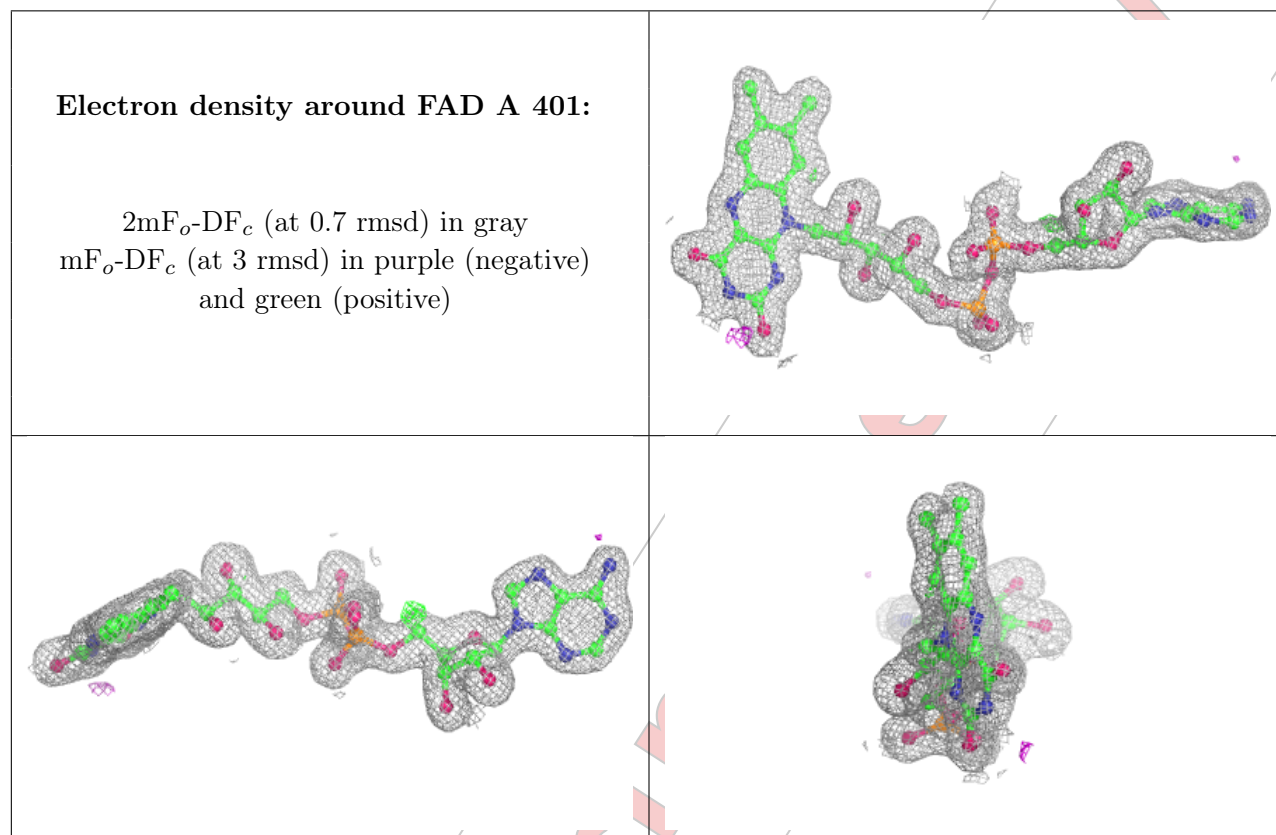
$2mF_o-DF_c$ (at 0.7 rmsd) in gray
 mF_o-DF_c (at 3 rmsd) in purple (negative)
 and green (positive)



Electron density around NAD A 402:

$2mF_o-DF_c$ (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.



Full wwPDB X-ray Structure Validation Report ⓘ

Feb 19, 2025 – 11:03 am GMT

PDB ID : 9IFZ
Title : FSP1 (tetrapod ancestor) bound to FAD
Deposited on : 2025-02-18
Resolution : 2.10 Å (reported)

This wwPDB validation report is for manuscript review

This is a Full wwPDB X-ray Structure Validation Report.

This report is produced by the wwPDB biocuration pipeline after annotation of the structure.

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 ⓘ symbol.

The types of validation reports are described at

<https://www.wwpdb.org/validation/2017/FAQs#types>.

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

MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (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	:	9.0.003 (Gargrove)
Density-Fitness	:	1.0.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)

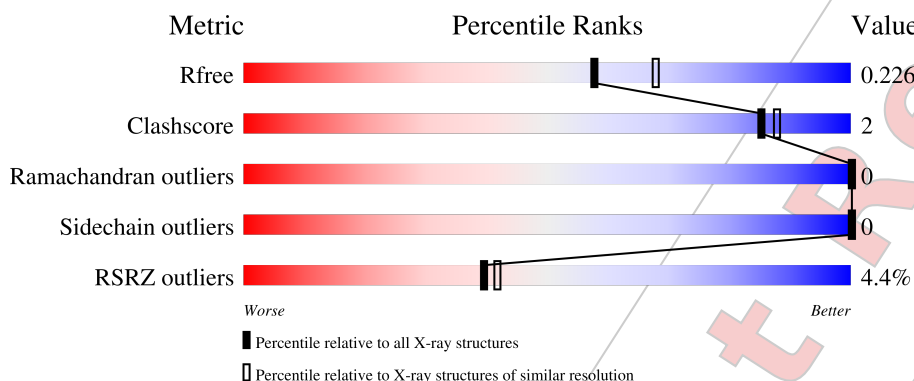
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 2.10 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	6234 (2.10-2.10)
Clashscore	180529	6893 (2.10-2.10)
Ramachandran outliers	177936	6839 (2.10-2.10)
Sidechain outliers	177891	6840 (2.10-2.10)
RSRZ outliers	164620	6234 (2.10-2.10)

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 $\leq 5\%$. 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	368	

The following table lists non-polymeric compounds, carbohydrate monomers and non-standard residues in protein, DNA, RNA chains that are outliers for geometric or electron-density-fit criteria:

Validation Pipeline (wwPDB-VP) : 2.41

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
3	PEG	A	402	-	-	X	-
3	PEG	A	403	-	-	X	-

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2 Entry composition [i](#)

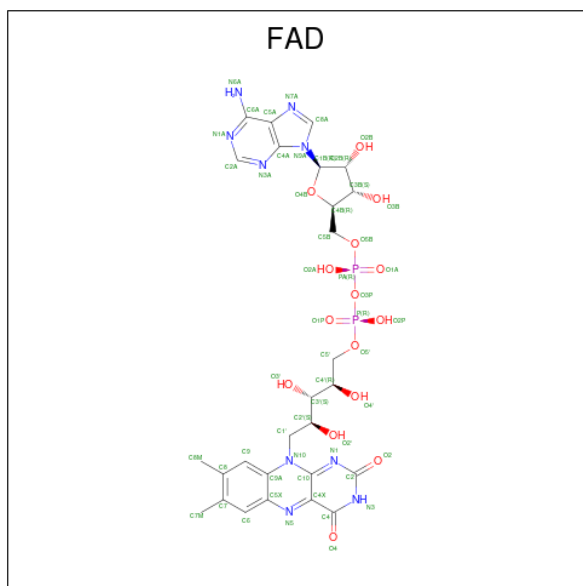
There are 4 unique types of molecules in this entry. The entry contains 2961 atoms, of which 0 are hydrogens and 0 are deuteriums.

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 FSP1 (Tetrapod ancestor).

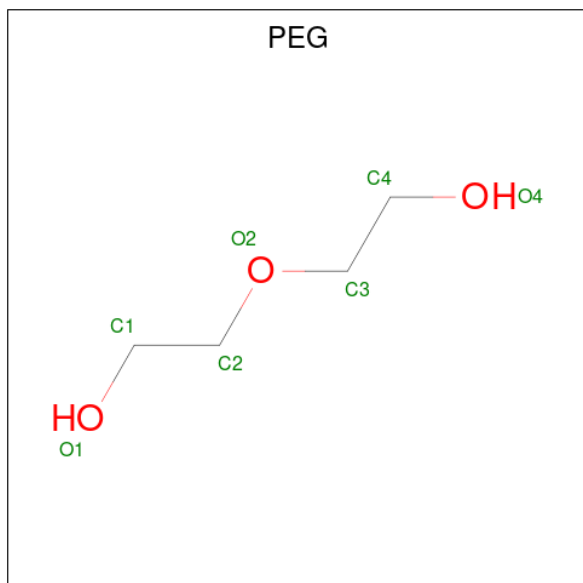
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	365	2769	1758	474	523	14	0	0	0

- Molecule 2 is FLAVIN-ADENINE DINUCLEOTIDE (three-letter code: FAD) (formula: $C_{27}H_{33}N_9O_{15}P_2$) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
			Total	C	N	O	P		
2	A	1	53	27	9	15	2	0	0

- Molecule 3 is DI(HYDROXYETHYL)ETHER (three-letter code: PEG) (formula: $C_4H_{10}O_3$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
3	A	1	Total	C	O	0	0
			7	4	3		
3	A	1	Total	C	O	0	0
			7	4	3		
3	A	1	Total	C	O	0	0
			7	4	3		

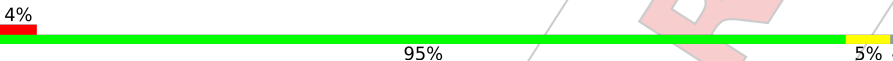
- Molecule 4 is water.

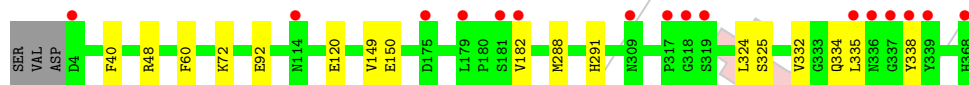
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
4	A	118	Total	O	0	0
			118	118		

3 Residue-property plots [i](#)

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: FSP1 (Tetrapod ancestor)

Chain A: 



4 Data and refinement statistics

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α , β , γ	43.09Å 81.51Å 57.69Å 90.00° 97.53° 90.00°	Depositor
Resolution (Å)	46.86 – 2.10 46.86 – 2.10	Depositor EDS
% Data completeness (in resolution range)	99.9 (46.86-2.10) 99.9 (46.86-2.10)	Depositor EDS
R_{merge}	0.19	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.95 (at 2.10Å)	Xtriage
Refinement program	REFMAC 5.8.0405	Depositor
R, R_{free}	0.180 , 0.227 0.188 , 0.226	Depositor DCC
R_{free} test set	1139 reflections (4.92%)	wwPDB-VP
Wilson B-factor (Å ²)	23.2	Xtriage
Anisotropy	0.122	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.34 , 34.8	EDS
L-test for twinning ²	$\langle L \rangle = 0.51$, $\langle L^2 \rangle = 0.35$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.95	EDS
Total number of atoms	2961	wwPDB-VP
Average B, all atoms (Å ²)	27.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 6.43% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical 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: FAD, PEG

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	0.42	2/2817 (0.1%)	0.68	0/3804

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	1

All (2) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	120	GLU	CD-OE1	5.49	1.31	1.25
1	A	120	GLU	CD-OE2	5.08	1.31	1.25

There are no bond angle outliers.

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	48	ARG	Sidechain

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	2769	0	2800	8	0
2	A	53	0	31	0	0
3	A	21	0	30	4	0
4	A	118	0	0	0	0
All	All	2961	0	2861	12	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 2.

All (12) 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)
3:A:402:PEG:O4	3:A:403:PEG:O1	1.77	0.78
3:A:402:PEG:C4	3:A:403:PEG:HO1	2.00	0.73
3:A:402:PEG:HO4	3:A:403:PEG:HO1	0.85	0.65
3:A:402:PEG:C4	3:A:403:PEG:O1	2.43	0.64
1:A:334:GLN:HA	1:A:338:TYR:O	2.01	0.59
1:A:72:LYS:NZ	1:A:92:GLU:OE1	2.39	0.51
1:A:288:MET:HG3	1:A:291:HIS:CE1	2.47	0.49
1:A:150:GLU:HG2	1:A:325:SER:O	2.16	0.45
1:A:182:VAL:HG13	1:A:332:VAL:HG13	1.99	0.44
1:A:40:PHE:O	1:A:60:PHE:HA	2.20	0.42
1:A:149:VAL:HG21	1:A:324:LEU:HD21	2.01	0.42
1:A:335:LEU:HD23	1:A:335:LEU:C	2.41	0.40

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	363/368 (99%)	352 (97%)	11 (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	298/302 (99%)	298 (100%)	0	100	100

There are no protein residues with a non-rotameric sidechain to report.

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	84	GLN

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](#)

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
2	FAD	A	401	-	53,58,58	0.67	1 (1%)	68,89,89	0.75	1 (1%)
3	PEG	A	404	-	6,6,6	0.36	0	5,5,5	0.28	0
3	PEG	A	402	-	6,6,6	0.13	0	5,5,5	0.14	0
3	PEG	A	403	-	6,6,6	0.18	0	5,5,5	0.17	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
2	FAD	A	401	-	-	6/30/50/50	0/6/6/6
3	PEG	A	404	-	-	1/4/4/4	-
3	PEG	A	402	-	-	2/4/4/4	-
3	PEG	A	403	-	-	2/4/4/4	-

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	401	FAD	C1'-C2'	-2.14	1.49	1.52

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	A	401	FAD	O4-C4-C4X	-2.02	121.23	126.60

There are no chirality outliers.

All (11) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	A	402	PEG	O2-C3-C4-O4
3	A	403	PEG	O2-C3-C4-O4
3	A	404	PEG	O2-C3-C4-O4
3	A	403	PEG	O1-C1-C2-O2
2	A	401	FAD	PA-O3P-P-O5'
2	A	401	FAD	O4B-C4B-C5B-O5B
3	A	402	PEG	O1-C1-C2-O2

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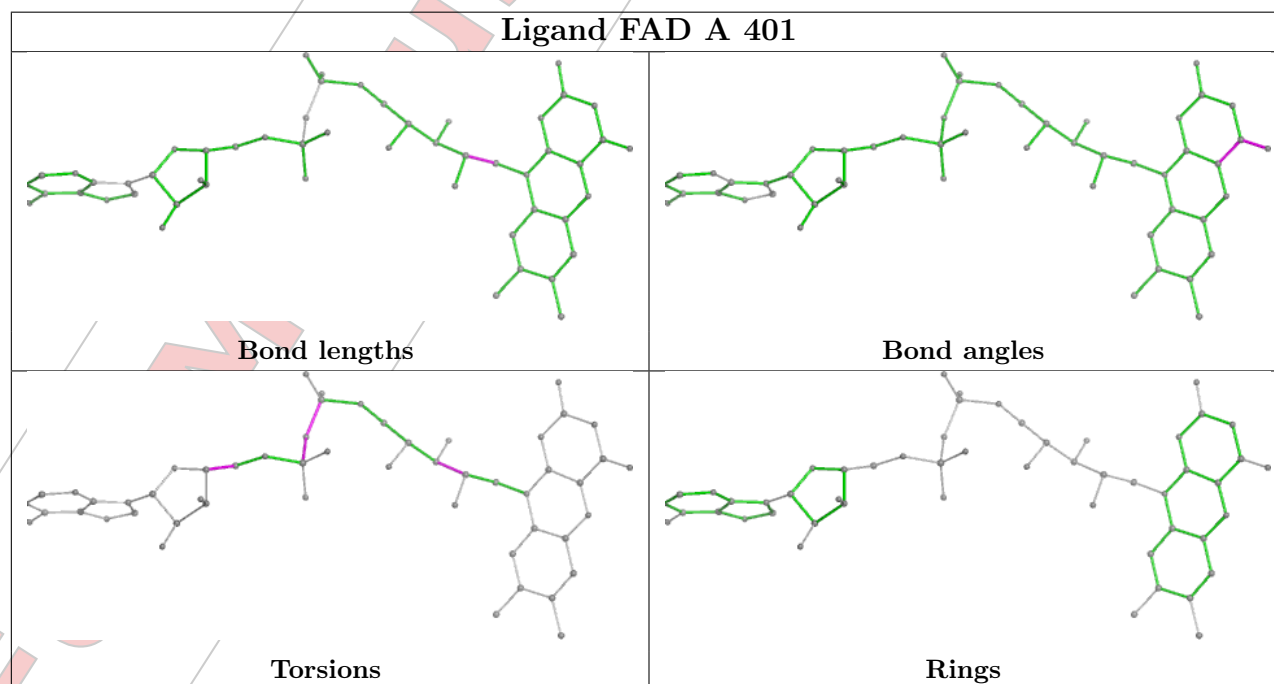
Mol	Chain	Res	Type	Atoms
2	A	401	FAD	P-O3P-PA-O1A
2	A	401	FAD	O2'-C2'-C3'-O3'
2	A	401	FAD	C3B-C4B-C5B-O5B
2	A	401	FAD	C1'-C2'-C3'-O3'

There are no ring outliers.

2 monomers are involved in 4 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	A	402	PEG	4	0
3	A	403	PEG	4	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 ⓘ

There are no such residues in this entry.

5.8 Polymer linkage issues ⓘ

There are no chain breaks in this entry.

For Manuscript Review

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, 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	365/368 (99%)	-0.09	16 (4%)	39 42	14, 24, 49, 75	0

All (16) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	339	TYR	4.1
1	A	338	TYR	4.0
1	A	336	ASN	3.9
1	A	182	VAL	3.9
1	A	335	LEU	3.8
1	A	337	GLY	3.6
1	A	318	GLY	3.3
1	A	4	ASP	3.3
1	A	368	HIS	2.8
1	A	179	LEU	2.7
1	A	317	PRO	2.4
1	A	181	SER	2.3
1	A	175	ASP	2.3
1	A	309	ASN	2.2
1	A	319	SER	2.2
1	A	114	ASN	2.1

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](#)

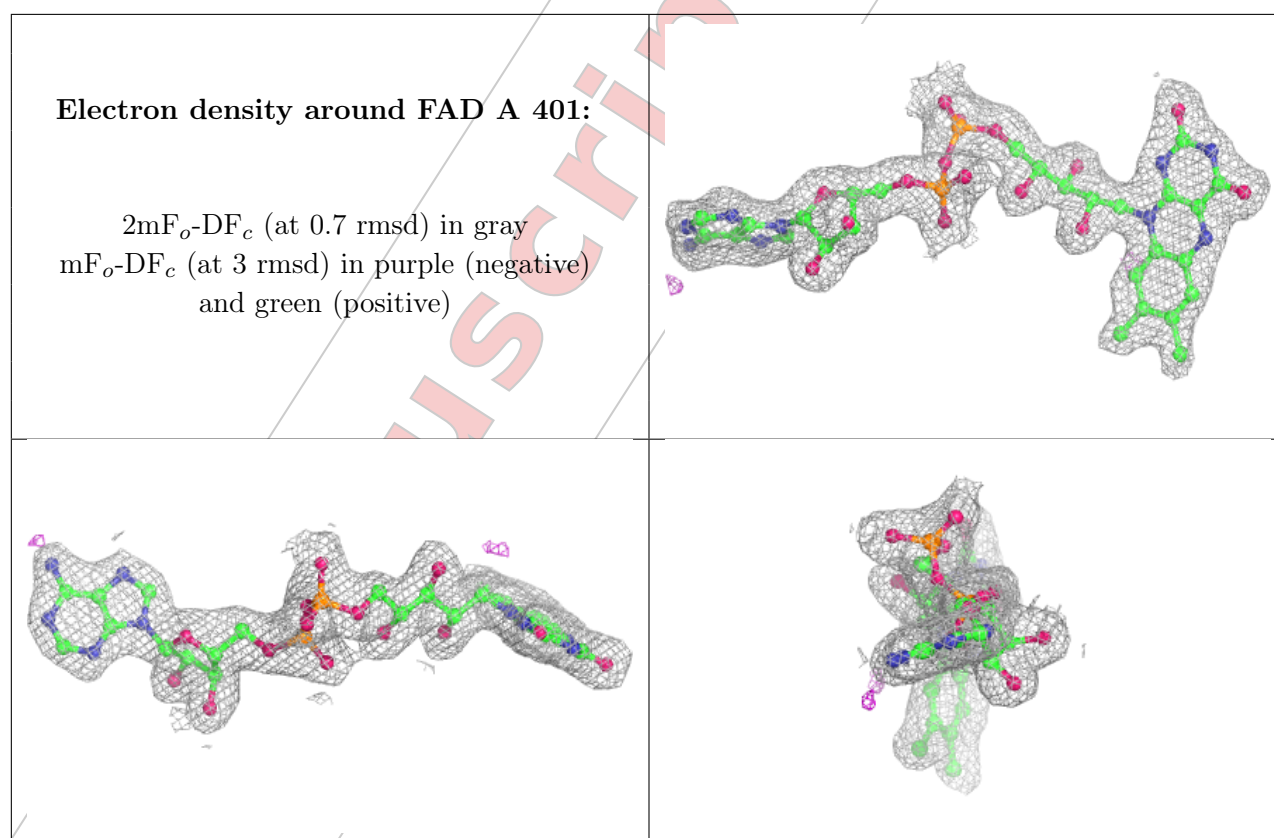
There are no monosaccharides in this entry.

6.4 Ligands ⓘ

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
3	PEG	A	404	7/7	0.82	0.18	48,53,61,63	0
3	PEG	A	403	7/7	0.87	0.14	44,45,48,48	0
3	PEG	A	402	7/7	0.91	0.13	40,41,45,45	0
2	FAD	A	401	53/53	0.98	0.05	14,18,26,31	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.



6.5 Other polymers ⓘ

There are no such residues in this entry.



Full wwPDB X-ray Structure Validation Report ⓘ

Mar 3, 2025 – 10:44 pm GMT

PDB ID : 9IG9
Title : FSP1 (tetrapod ancestor) bound to FAD and NAD⁺ and compound 3
Deposited on : 2025-02-19
Resolution : 1.94 Å (reported)

This wwPDB validation report is for manuscript review

This is a Full wwPDB X-ray Structure Validation Report.

This report is produced by the wwPDB biocuration pipeline after annotation of the structure.

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 ⓘ symbol.

The types of validation reports are described at

<https://www.wwpdb.org/validation/2017/FAQs#types>.

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

MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (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	:	9.0.003 (Gargrove)
Density-Fitness	:	1.0.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)

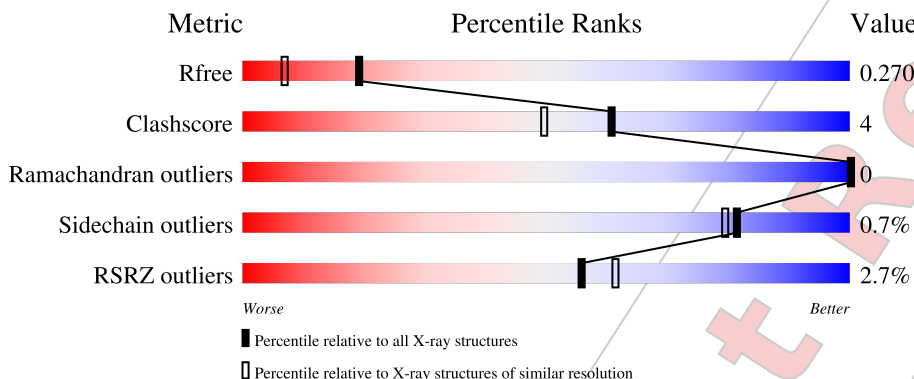
1 Overall quality at a glance i

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 1.94 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	1306 (1.94-1.94)
Clashscore	180529	1400 (1.94-1.94)
Ramachandran outliers	177936	1387 (1.94-1.94)
Sidechain outliers	177891	1387 (1.94-1.94)
RSRZ outliers	164620	1306 (1.94-1.94)

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 $\leq 5\%$. 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	368	% 86% 12%
1	B	368	5% 90% 9%

2 Entry composition [i](#)

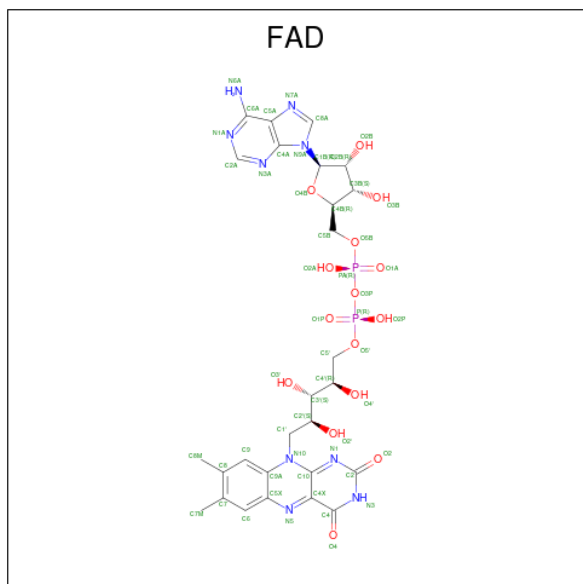
There are 9 unique types of molecules in this entry. The entry contains 6003 atoms, of which 0 are hydrogens and 0 are deuteriums.

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 FSP1 (tetrapod ancestor).

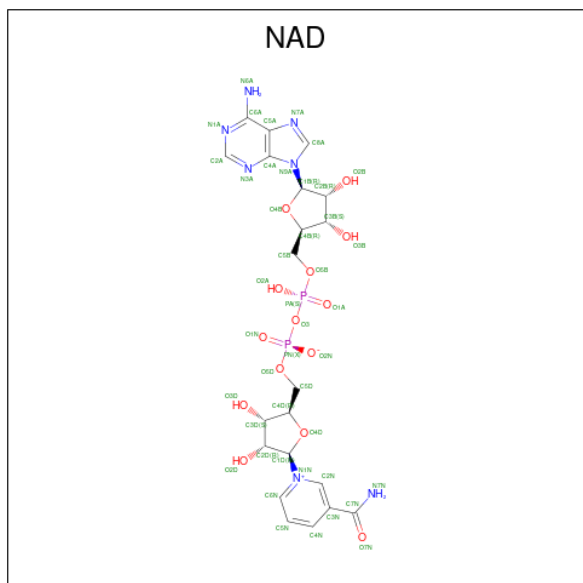
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	364	Total	C	N	O	S	0	2	0
			2775	1761	473	527	14			
1	B	365	Total	C	N	O	S	0	1	0
			2773	1759	473	527	14			

- Molecule 2 is FLAVIN-ADENINE DINUCLEOTIDE (three-letter code: FAD) (formula: $C_{27}H_{33}N_9O_{15}P_2$).



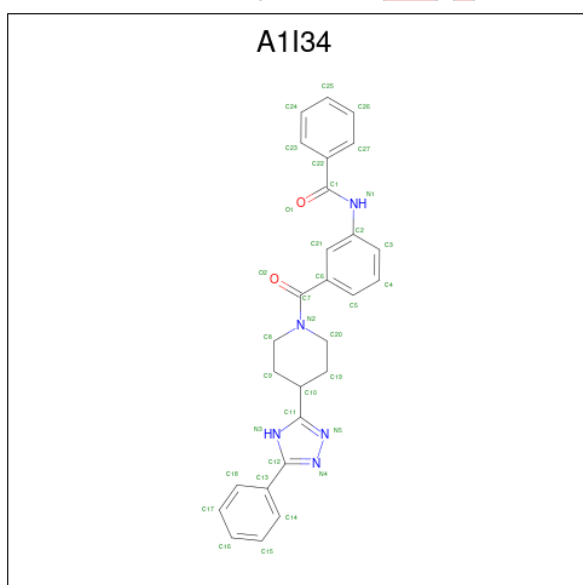
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
2	A	1	Total	C	N	O	P	0	0
			53	27	9	15	2		
2	B	1	Total	C	N	O	P	0	0
			53	27	9	15	2		

- Molecule 3 is NICOTINAMIDE-ADENINE-DINUCLEOTIDE (three-letter code: NAD) (formula: $C_{21}H_{27}N_7O_{14}P_2$).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	
3	A	1	Total	C	N	O	P	0	0
			44	21	7	14	2		
3	B	1	Total	C	N	O	P	0	0
			44	21	7	14	2		

- Molecule 4 is {N}-[3-[4-(5-phenyl-4 {H}-1,2,4-triazol-3-yl)piperidin-1-yl]carbonylphenyl]benzamide (three-letter code: A1I34) (formula: $C_{27}H_{25}N_5O_2$) (labeled as "Ligand of Interest" by depositor).



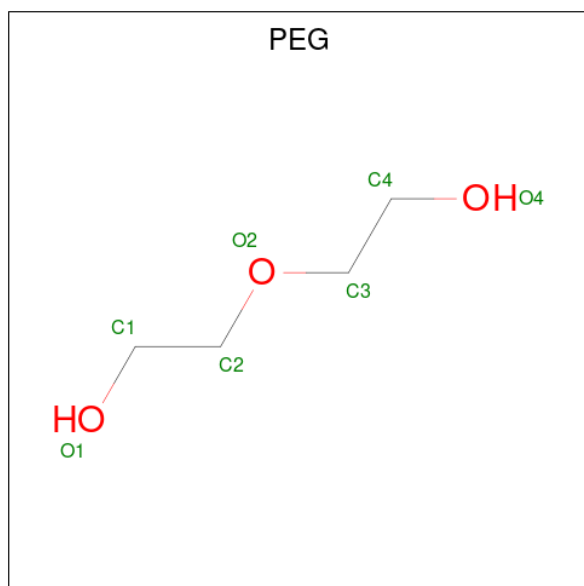
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
4	A	1	Total	C	N	O	0	0
			34	27	5	2		

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Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
4	B	1	Total	C	N	O	0	0
			34	27	5	2		

- Molecule 5 is DI(HYDROXYETHYL)ETHER (three-letter code: PEG) (formula: $C_4H_{10}O_3$).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
5	A	1	Total	C	O	0	0
			7	4	3		

- Molecule 6 is CALCIUM ION (three-letter code: CA) (formula: Ca).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
6	A	1	Total	Ca	0	0
			1	1		

- Molecule 7 is CHLORIDE ION (three-letter code: CL) (formula: Cl).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
7	A	1	Total	Cl	0	0
			1	1		

- Molecule 8 is MAGNESIUM ION (three-letter code: MG) (formula: Mg).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
8	A	1	Total	Mg	0	0
			1	1		

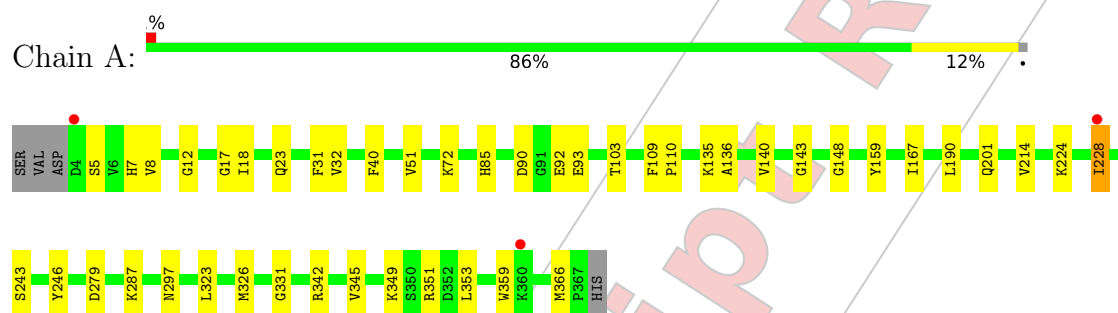
- Molecule 9 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
9	A	112	Total 112	O 112	0	0
9	B	71	Total 71	O 71	0	0

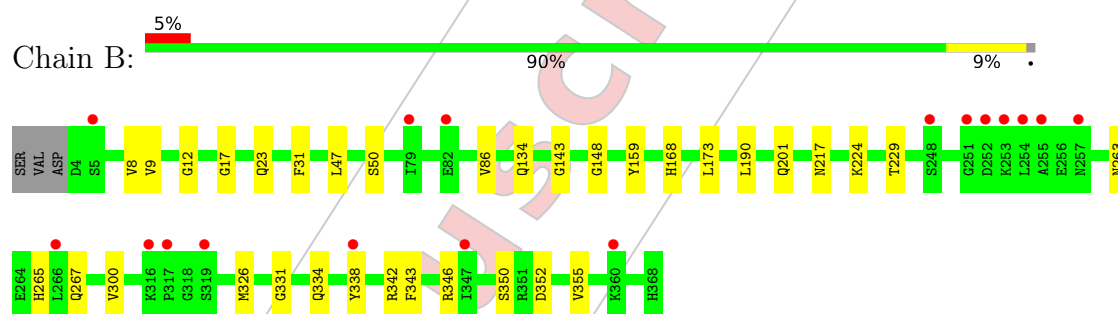
3 Residue-property plots [i](#)

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: FSP1 (tetrapod ancestor)



- Molecule 1: FSP1 (tetrapod ancestor)



4 Data and refinement statistics

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α , β , γ	43.85Å 79.33Å 113.37Å 90.00° 99.08° 90.00°	Depositor
Resolution (Å)	43.34 – 1.94 43.34 – 1.94	Depositor EDS
% Data completeness (in resolution range)	93.4 (43.34-1.94) 93.6 (43.34-1.94)	Depositor EDS
R_{merge}	0.12	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.14 (at 1.94Å)	Xtriage
Refinement program	REFMAC 5.8.0405	Depositor
R, R_{free}	0.215 , 0.265 0.227 , 0.270	Depositor DCC
R_{free} test set	2734 reflections (4.77%)	wwPDB-VP
Wilson B-factor (Å ²)	21.4	Xtriage
Anisotropy	0.166	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.34 , 37.8	EDS
L-test for twinning ²	$\langle L \rangle = 0.47$, $\langle L^2 \rangle = 0.30$	Xtriage
Estimated twinning fraction	0.038 for h,-k,-h-l	Xtriage
F_o, F_c correlation	0.93	EDS
Total number of atoms	6003	wwPDB-VP
Average B, all atoms (Å ²)	29.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.11% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical 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: PEG, FAD, MG, NAD, CL, A1I34, CA

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	0.45	0/2822	0.74	0/3811
1	B	0.40	0/2820	0.74	0/3808
All	All	0.43	0/5642	0.74	0/7619

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 [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	2775	0	2807	26	0
1	B	2773	0	2801	20	0
2	A	53	0	31	1	0
2	B	53	0	31	0	0
3	A	44	0	26	0	0
3	B	44	0	26	0	0
4	A	34	0	0	1	0
4	B	34	0	0	0	0
5	A	7	0	10	0	0
6	A	1	0	0	0	0
7	A	1	0	0	0	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
8	A	1	0	0	0	0
9	A	112	0	0	1	0
9	B	71	0	0	0	0
All	All	6003	0	5732	47	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.

All (47) 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:23:GLN:HE22	1:A:297:ASN:HA	1.51	0.73
1:B:23:GLN:HB2	1:B:300:VAL:HG11	1.73	0.70
1:A:85:HIS:ND1	1:A:93:GLU:OE1	2.23	0.70
1:B:263:ASN:HD21	1:B:267:GLN:HB2	1.57	0.69
1:A:167:ILE:HD11	1:A:228:ILE:CD1	2.23	0.68
1:B:331:GLY:O	1:B:342:ARG:HA	1.98	0.64
1:A:140:VAL:HG21	1:A:228:ILE:HD11	1.80	0.62
1:A:7:HIS:NE2	1:A:32:VAL:HG23	2.15	0.60
1:B:47:LEU:O	1:B:50:SER:HB2	2.00	0.60
1:B:143:GLY:O	1:B:148:GLY:HA3	2.06	0.56
1:B:190:LEU:HG	1:B:326:MET:HE1	1.87	0.56
1:A:323:LEU:HD22	1:A:345:VAL:HG22	1.92	0.52
1:B:134:GLN:HG2	1:B:159:TYR:OH	2.09	0.52
1:B:346:ARG:HA	1:B:350:SER:HB2	1.91	0.51
4:A:403:A1I34:C8	4:A:403:A1I34:C21	2.91	0.49
1:A:228:ILE:O	1:A:228:ILE:HG12	2.12	0.49
1:B:8:VAL:O	1:B:31:PHE:HA	2.13	0.49
1:A:351:ARG:HB2	9:A:557:HOH:O	2.13	0.49
1:A:103:THR:O	2:A:401:FAD:H8A	2.12	0.48
1:B:334:GLN:HA	1:B:338:TYR:O	2.13	0.48
1:B:201:GLN:HB3	1:B:224:LYS:HG3	1.97	0.46
1:A:51[B]:VAL:HG13	1:A:159:TYR:HE2	1.80	0.46
1:B:190:LEU:CG	1:B:326:MET:HE1	2.45	0.46
1:A:90:ASP:OD1	1:A:92:GLU:HB2	2.15	0.46
1:B:12:GLY:O	1:B:17:GLY:HA3	2.16	0.45
1:A:143:GLY:O	1:A:148:GLY:HA3	2.16	0.45
1:A:109:PHE:CG	1:A:110:PRO:HA	2.51	0.45
1:B:190:LEU:HG	1:B:326:MET:CE	2.46	0.45
1:B:168:HIS:CD2	1:B:173:LEU:HD23	2.52	0.44
1:B:263:ASN:OD1	1:B:265:HIS:HB2	2.17	0.44

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:243:SER:HA	1:A:246:TYR:CZ	2.53	0.43
1:A:12:GLY:O	1:A:17:GLY:HA3	2.19	0.43
1:A:135:LYS:HB2	1:A:214:VAL:HG21	2.01	0.43
1:A:167:ILE:HD11	1:A:228:ILE:HD12	1.99	0.43
1:A:18:ILE:HD12	1:A:40:PHE:CD1	2.54	0.43
1:A:8:VAL:O	1:A:31:PHE:HA	2.20	0.42
1:A:190:LEU:HG	1:A:326:MET:HE1	2.00	0.42
1:A:349:LYS:O	1:A:353:LEU:N	2.50	0.41
1:A:136:ALA:HB2	1:A:214:VAL:HG22	2.02	0.41
1:A:331:GLY:O	1:A:342:ARG:HA	2.21	0.41
1:B:217:ASN:HA	1:B:229:THR:CG2	2.51	0.41
1:B:201:GLN:HB3	1:B:224:LYS:CG	2.51	0.41
1:B:352:ASP:O	1:B:355:VAL:HG13	2.21	0.40
1:A:279:ASP:HA	1:A:287:LYS:HD3	2.02	0.40
1:A:201:GLN:HB3	1:A:224:LYS:HG3	2.03	0.40
1:B:9:VAL:HG11	1:B:86:VAL:HG21	2.03	0.40
1:A:359:TRP:CD1	1:A:366:MET:HE2	2.57	0.40

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	364/368 (99%)	353 (97%)	11 (3%)	0	100	100
1	B	364/368 (99%)	352 (97%)	12 (3%)	0	100	100
All	All	728/736 (99%)	705 (97%)	23 (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	300/302 (99%)	297 (99%)	3 (1%)	73	68
1	B	299/302 (99%)	298 (100%)	1 (0%)	91	91
All	All	599/604 (99%)	595 (99%)	4 (1%)	81	79

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

Mol	Chain	Res	Type
1	A	5	SER
1	A	72	LYS
1	A	228	ILE
1	B	343	PHE

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

Mol	Chain	Res	Type
1	A	23	GLN
1	B	168	HIS

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

Of 10 ligands modelled in this entry, 3 are monoatomic - leaving 7 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	FAD	B	401	-	53,58,58	0.69	0	68,89,89	0.71	2 (2%)
4	A1I34	B	403	-	35,38,38	0.53	0	49,52,52	0.97	2 (4%)
3	NAD	A	402	-	42,48,48	0.78	1 (2%)	50,73,73	0.96	2 (4%)
4	A1I34	A	403	-	35,38,38	0.48	0	49,52,52	0.73	0
5	PEG	A	404	-	6,6,6	0.45	0	5,5,5	0.29	0
3	NAD	B	402	-	42,48,48	0.69	1 (2%)	50,73,73	0.73	1 (2%)
2	FAD	A	401	-	53,58,58	0.69	0	68,89,89	0.84	1 (1%)

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	FAD	B	401	-	-	4/30/50/50	0/6/6/6
4	A1I34	B	403	-	-	6/20/34/34	1/5/5/5
3	NAD	A	402	-	-	1/26/62/62	0/5/5/5
4	A1I34	A	403	-	-	6/20/34/34	1/5/5/5
5	PEG	A	404	-	-	4/4/4/4	-
3	NAD	B	402	-	-	1/26/62/62	0/5/5/5
2	FAD	A	401	-	-	5/30/50/50	0/6/6/6

All (2) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	A	402	NAD	C2N-N1N	2.96	1.38	1.35
3	B	402	NAD	C2N-N1N	2.39	1.37	1.35

All (8) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	A	401	FAD	C5A-C6A-N6A	3.64	125.88	120.35
4	B	403	A1I34	C10-C11-N3	3.47	131.68	125.08
3	A	402	NAD	C5A-C6A-N6A	3.32	125.39	120.35
4	B	403	A1I34	C11-N5-N4	2.39	111.70	106.64
2	B	401	FAD	C5A-C6A-N6A	2.31	123.87	120.35
3	B	402	NAD	C5A-C6A-N6A	2.26	123.79	120.35
3	A	402	NAD	C6N-N1N-C2N	-2.25	119.93	121.97
2	B	401	FAD	C4-N3-C2	-2.03	121.89	125.64

There are no chirality outliers.

All (27) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	B	401	FAD	O4B-C4B-C5B-O5B
4	A	403	A1I34	N3-C12-C13-C14
4	A	403	A1I34	N3-C12-C13-C18
4	A	403	A1I34	N4-C12-C13-C14
4	A	403	A1I34	N4-C12-C13-C18
4	A	403	A1I34	C6-C7-N2-C20
5	A	404	PEG	O2-C3-C4-O4
2	B	401	FAD	C3B-C4B-C5B-O5B
5	A	404	PEG	O1-C1-C2-O2
4	A	403	A1I34	O2-C7-N2-C20
5	A	404	PEG	C4-C3-O2-C2
4	B	403	A1I34	O2-C7-N2-C8
4	B	403	A1I34	N3-C12-C13-C18
4	B	403	A1I34	N4-C12-C13-C14
5	A	404	PEG	C1-C2-O2-C3
2	B	401	FAD	O2'-C2'-C3'-O3'
2	A	401	FAD	O4B-C4B-C5B-O5B
4	B	403	A1I34	N4-C12-C13-C18
4	B	403	A1I34	N3-C12-C13-C14
2	A	401	FAD	P-O3P-PA-O1A
2	A	401	FAD	P-O3P-PA-O2A
2	A	401	FAD	O2'-C2'-C3'-O3'
4	B	403	A1I34	C6-C7-N2-C8
3	A	402	NAD	O4B-C4B-C5B-O5B
3	B	402	NAD	O4B-C4B-C5B-O5B
2	A	401	FAD	C1'-C2'-C3'-O3'
2	B	401	FAD	C1'-C2'-C3'-O3'

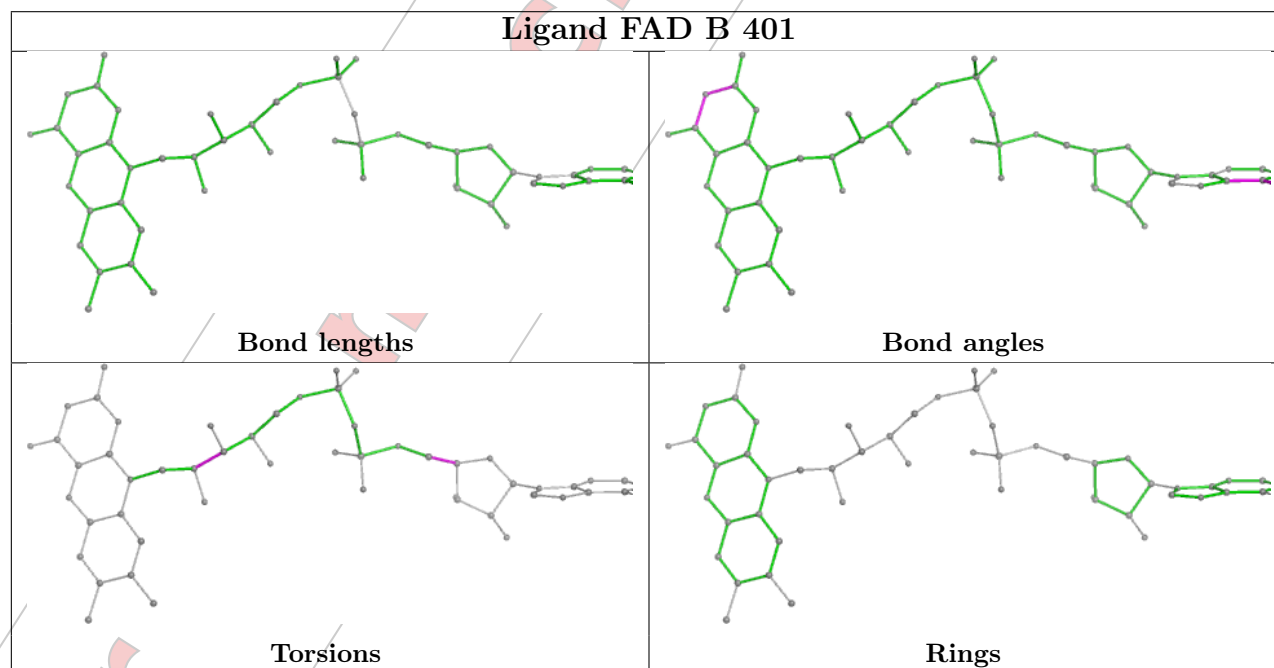
All (2) ring outliers are listed below:

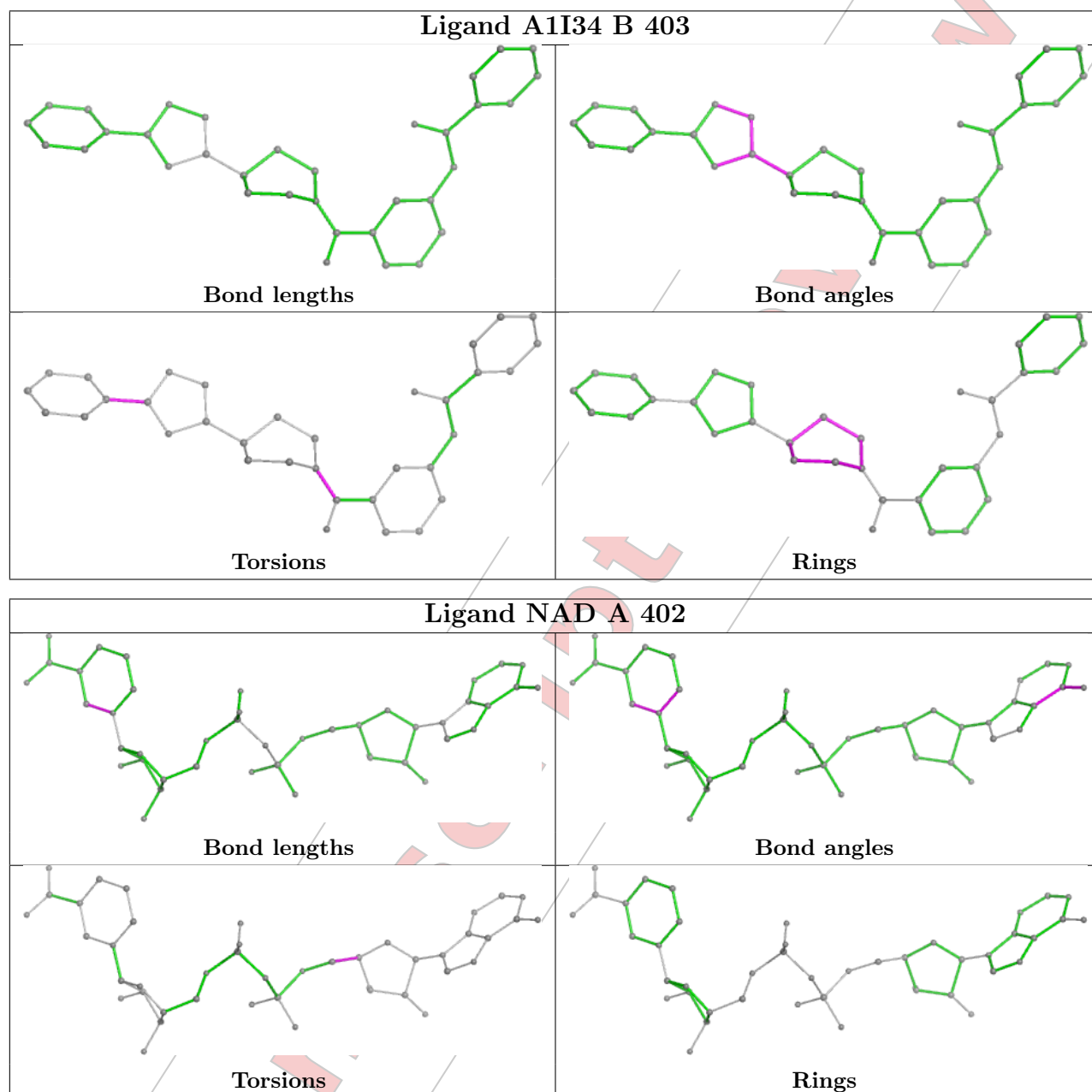
Mol	Chain	Res	Type	Atoms
4	B	403	A1I34	C10-C19-C20-C8-C9-N2
4	A	403	A1I34	C10-C19-C20-C8-C9-N2

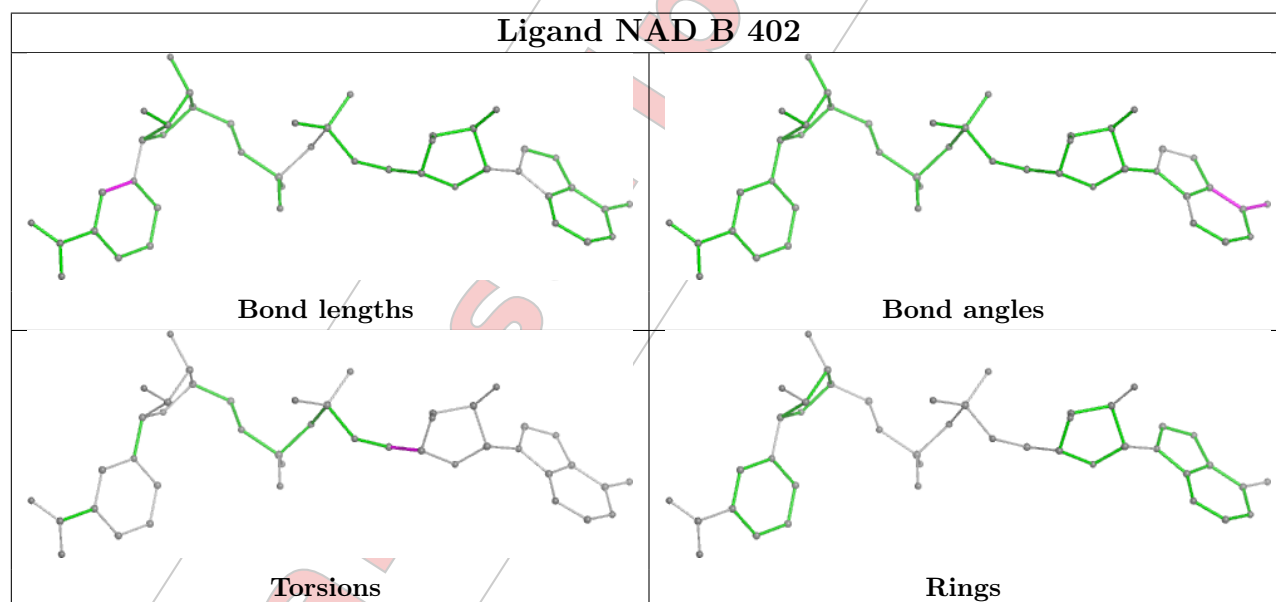
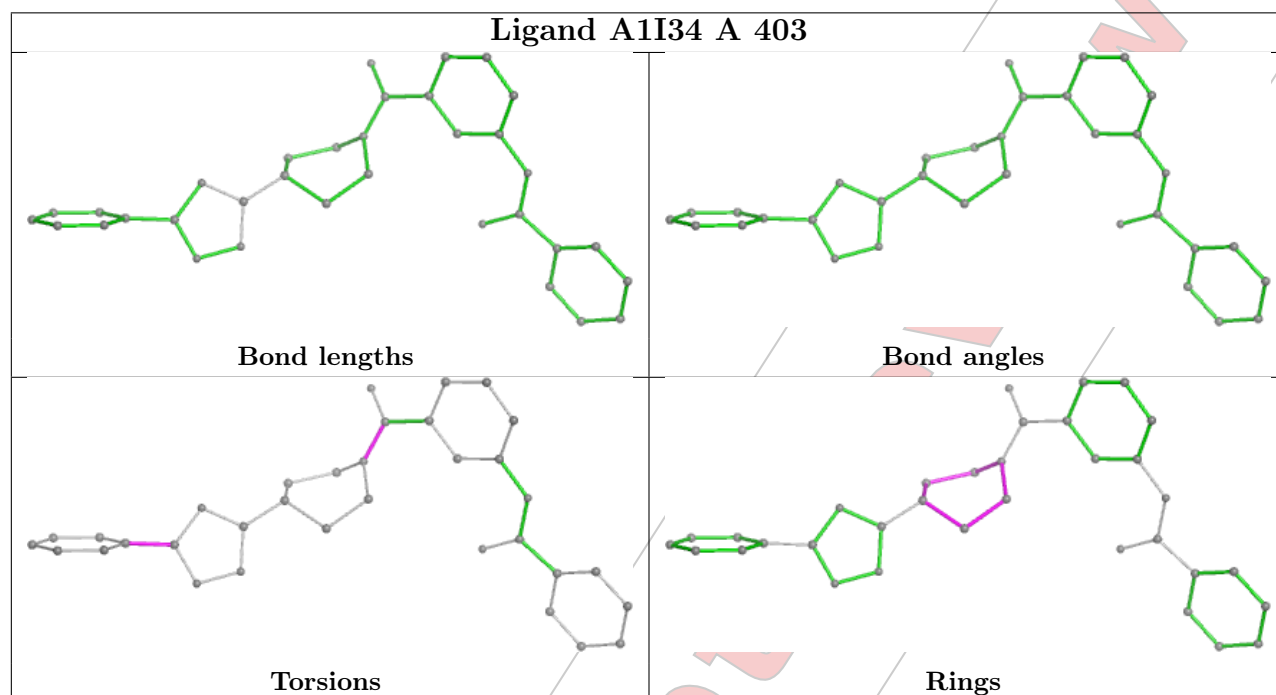
2 monomers are involved in 2 short contacts:

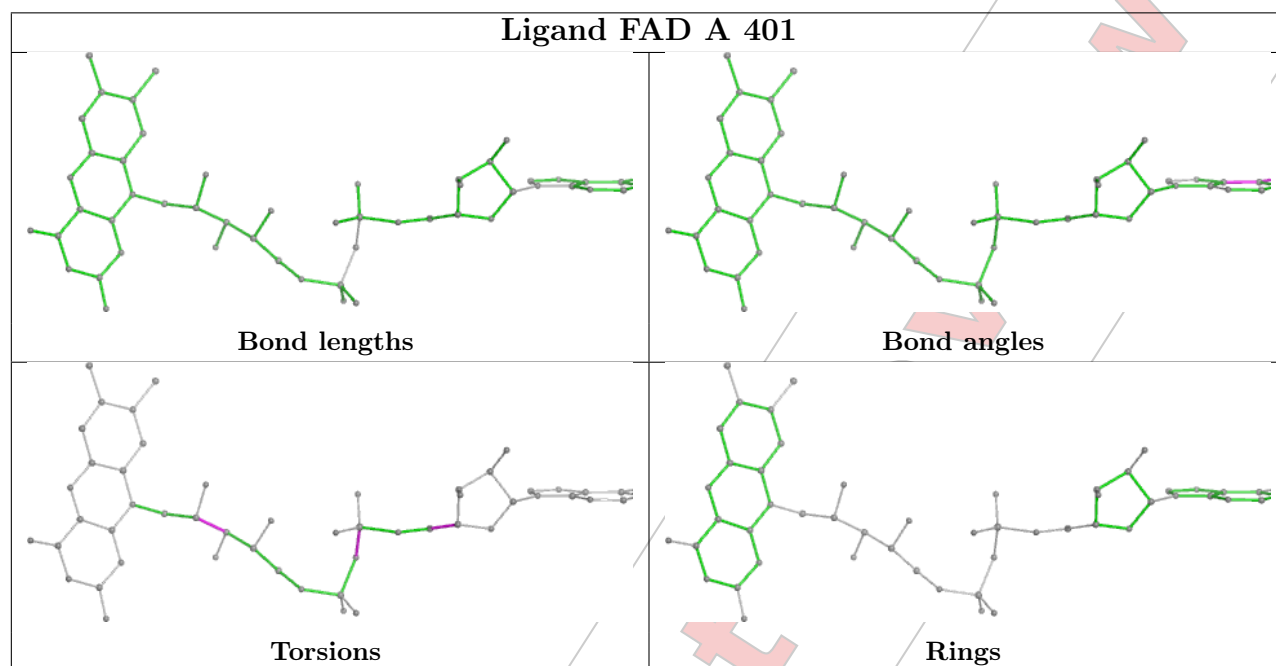
Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	A	403	A1I34	1	0
2	A	401	FAD	1	0

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

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	364/368 (98%)	-0.11	3 (0%) 82 87	9, 22, 39, 66	2 (0%)
1	B	365/368 (99%)	0.47	17 (4%) 37 41	12, 31, 52, 72	1 (0%)
All	All	729/736 (99%)	0.18	20 (2%) 56 61	9, 27, 49, 72	3 (0%)

All (20) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	B	253	LYS	3.5
1	B	252	ASP	3.1
1	B	251	GLY	2.9
1	B	248	SER	2.9
1	B	347	ILE	2.8
1	B	316	LYS	2.6
1	B	317	PRO	2.5
1	B	254	LEU	2.5
1	B	338	TYR	2.4
1	B	79	ILE	2.4
1	A	228	ILE	2.4
1	A	4	ASP	2.4
1	B	266	LEU	2.3
1	A	360	LYS	2.2
1	B	360	LYS	2.2
1	B	5	SER	2.1
1	B	257	ASN	2.1
1	B	82	GLU	2.1
1	B	255	ALA	2.0
1	B	319	SER	2.0

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](#)

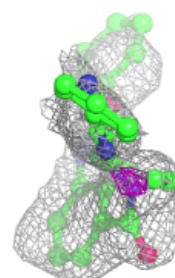
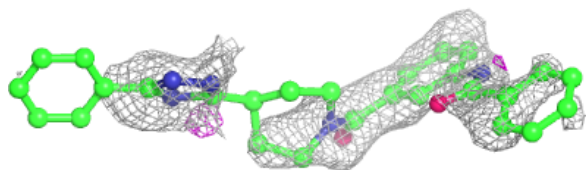
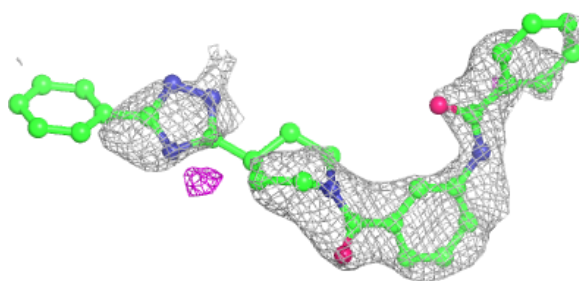
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95th percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists 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	A1I34	B	403	34/34	0.82	0.19	44,67,99,105	0
5	PEG	A	404	7/7	0.83	0.15	29,38,46,49	0
4	A1I34	A	403	34/34	0.88	0.12	29,46,58,62	0
8	MG	A	407	1/1	0.90	0.16	35,35,35,35	0
6	CA	A	405	1/1	0.95	0.07	42,42,42,42	0
3	NAD	B	402	44/44	0.95	0.07	19,24,27,31	0
2	FAD	B	401	53/53	0.96	0.07	17,22,26,28	0
7	CL	A	406	1/1	0.97	0.09	39,39,39,39	0
2	FAD	A	401	53/53	0.97	0.05	13,15,18,20	0
3	NAD	A	402	44/44	0.98	0.05	12,17,22,24	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.

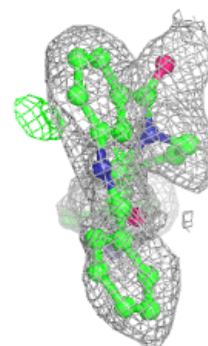
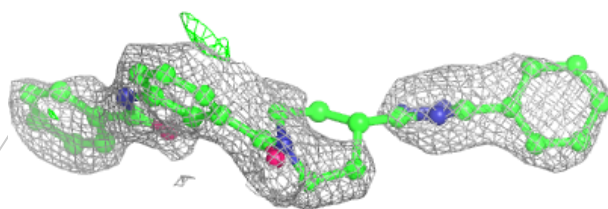
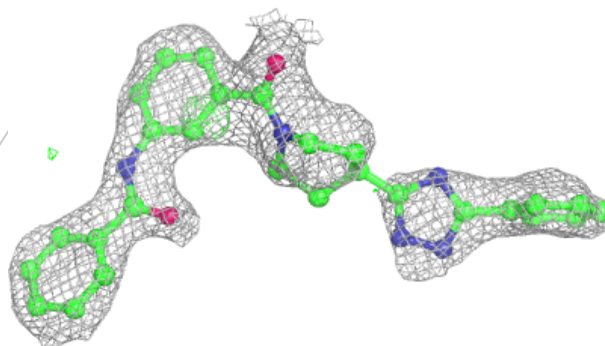
Electron density around A1I34 B 403:

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



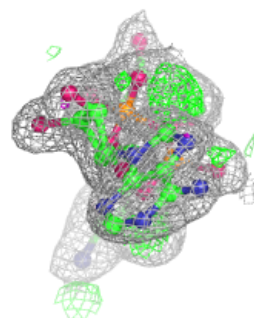
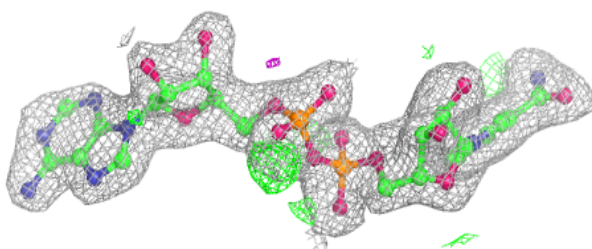
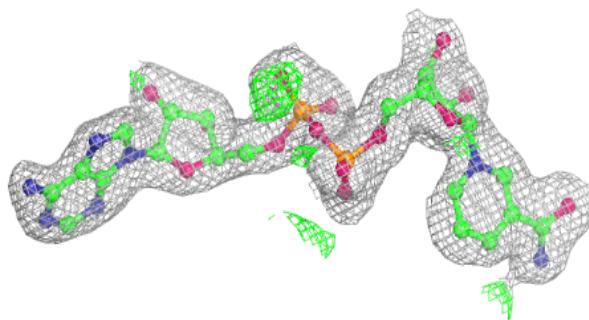
Electron density around A1I34 A 403:

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



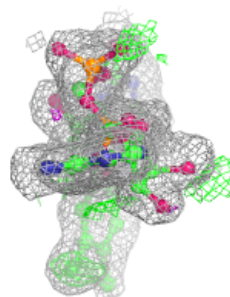
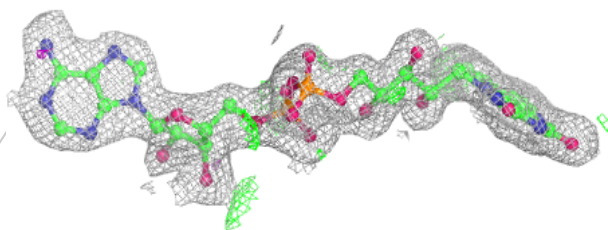
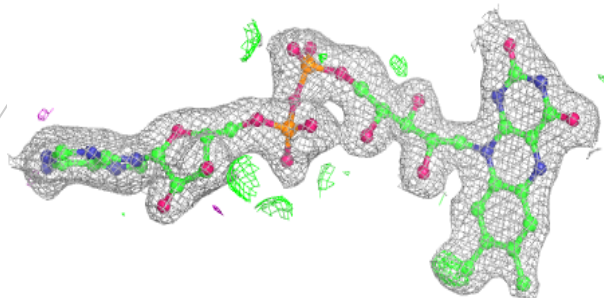
Electron density around NAD B 402:

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



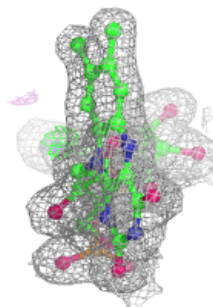
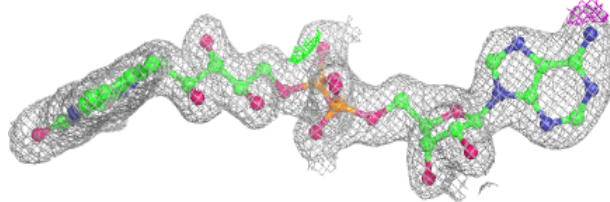
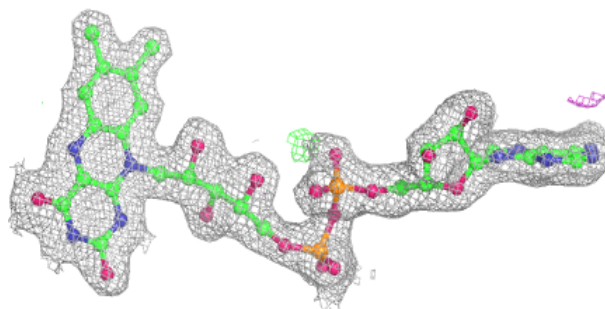
Electron density around FAD B 401:

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



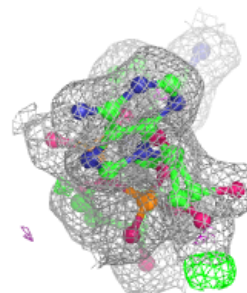
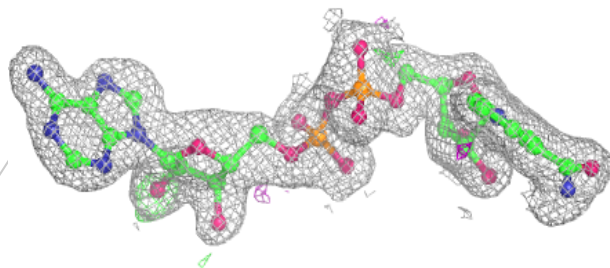
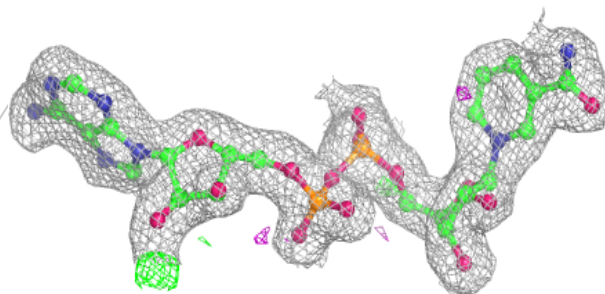
Electron density around FAD A 401:

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



Electron density around NAD A 402:

$2mF_o-DF_c$ (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.

For Manuscript Review



Full wwPDB X-ray Structure Validation Report ⓘ

Apr 4, 2025 – 05:35 pm BST

PDB ID : 9QRT / pdb_00009qrt
Title : FSP1 (tetrapod ancestor; L323A mutant) bound to FAD and NAD⁺ and compound 2
Deposited on : 2025-04-04
Resolution : 2.40 Å (reported)

This wwPDB validation report is for manuscript review

This is a Full wwPDB X-ray Structure Validation Report.

This report is produced by the wwPDB biocuration pipeline after annotation of the structure.

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 ⓘ 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 ⓘ](#)) were used in the production of this report:

MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (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	:	9.0.003 (Gargrove)
Density-Fitness	:	1.0.11
Ideal geometry (proteins)	:	Engh & Huber (2001)

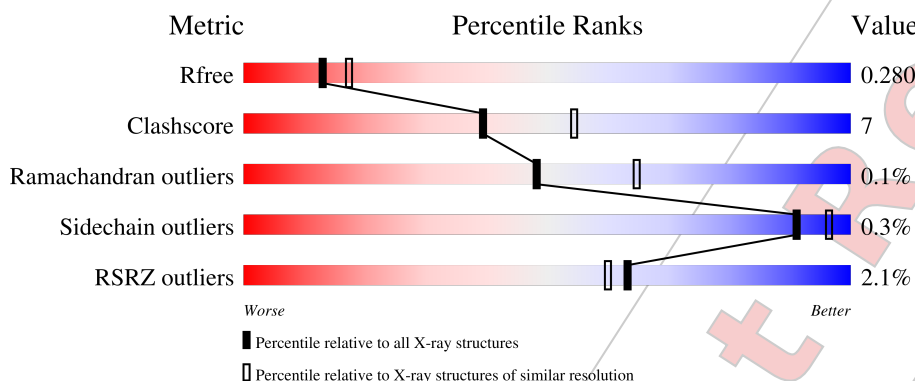
1 Overall quality at a glance i

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 2.40 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	4642 (2.40-2.40)
Clashscore	180529	5218 (2.40-2.40)
Ramachandran outliers	177936	5158 (2.40-2.40)
Sidechain outliers	177891	5159 (2.40-2.40)
RSRZ outliers	164620	4642 (2.40-2.40)

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 $\leq 5\%$. 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	368	% 86% 13%
1	B	368	3% 86% 12%

Ideal geometry (DNA, RNA) : Parkinson et al. (1996)

Validation Pipeline (wwPDB-VP) : 2.42

2 Entry composition [i](#)

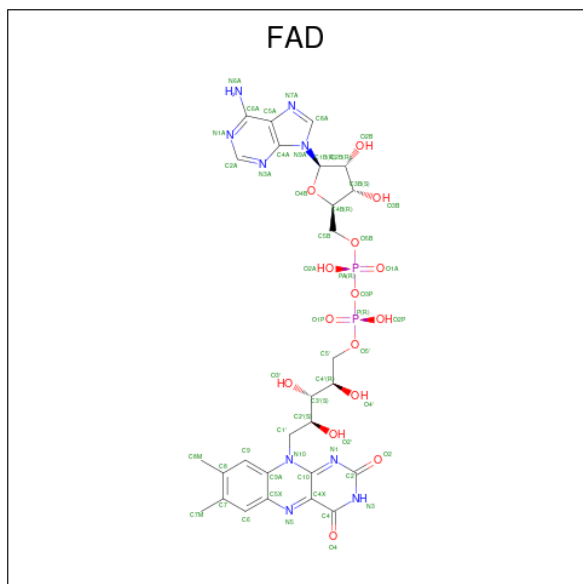
There are 6 unique types of molecules in this entry. The entry contains 5923 atoms, of which 0 are hydrogens and 0 are deuteriums.

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 FSP1 (tetrapod ancestor; L323A mutant) bound to FAD and NAD⁺ and compound 2.

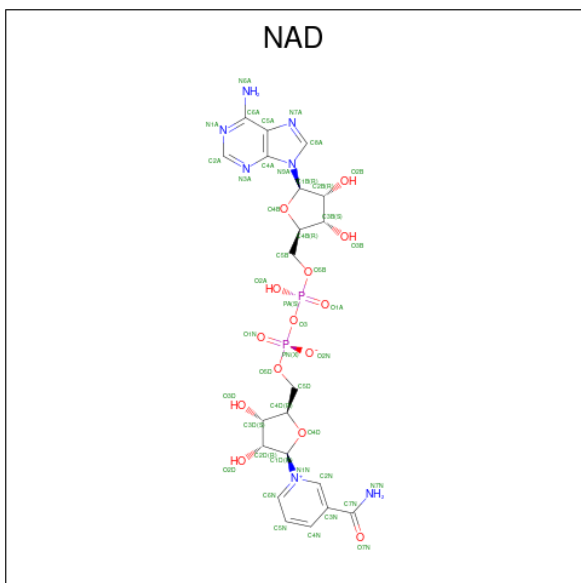
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	364	Total	C	N	O	S	0	0	0
			2759	1750	471	524	14			
1	B	365	Total	C	N	O	S	0	1	0
			2775	1759	475	527	14			

- Molecule 2 is FLAVIN-ADENINE DINUCLEOTIDE (CCD ID: FAD) (formula: C₂₇H₃₃N₉O₁₅P₂).



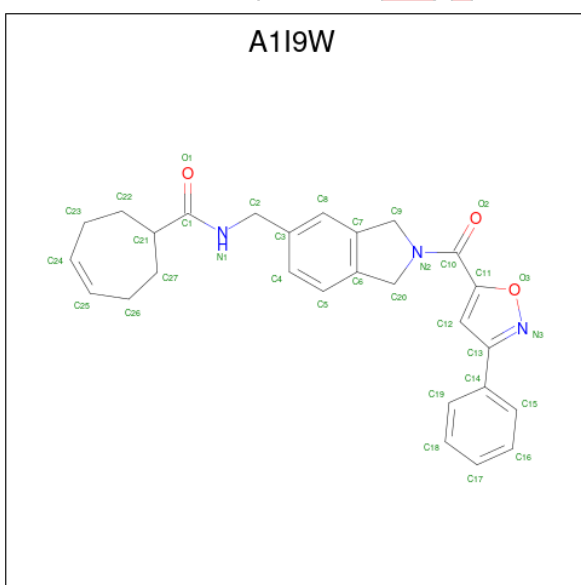
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
2	A	1	Total	C	N	O	P	0	0
			53	27	9	15	2		
2	B	1	Total	C	N	O	P	0	0
			53	27	9	15	2		

- Molecule 3 is NICOTINAMIDE-ADENINE-DINUCLEOTIDE (CCD ID: NAD) (formula: C₂₁H₂₇N₇O₁₄P₂).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	
3	A	1	Total	C	N	O	P	0	0
			44	21	7	14	2		
3	B	1	Total	C	N	O	P	0	0
			44	21	7	14	2		

- Molecule 4 is {N}-[[2-[(3-phenyl-1,2-oxazol-5-yl)carbonyl]-1,3-dihydroisindol-5-yl]methyl]cyclohept-4-ene-1-carboxamide (CCD ID: A1I9W) (formula: C₂₇H₂₇N₃O₃) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
4	A	1	Total	C	N	O	0	0
			33	27	3	3		

- Molecule 5 is CALCIUM ION (CCD ID: CA) (formula: Ca).

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

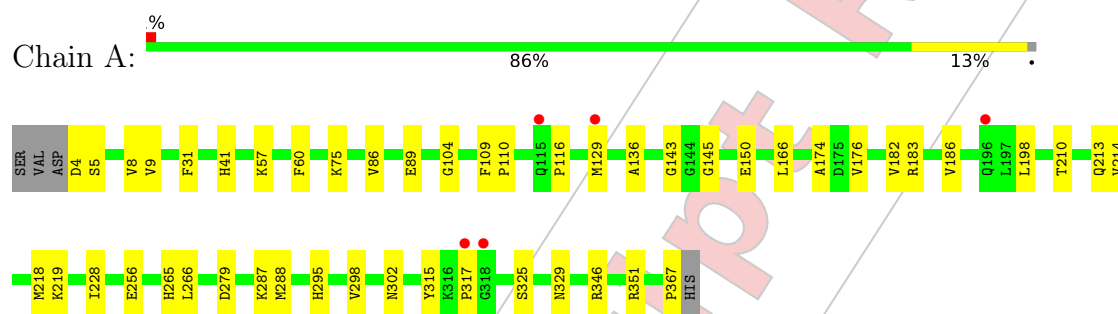
- Molecule 6 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
6	A	103	Total 103	O 103	0	0
6	B	58	Total 58	O 58	0	0

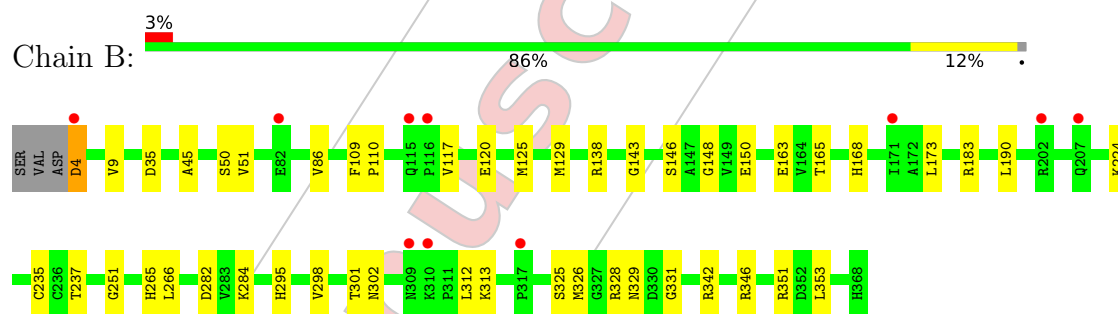
3 Residue-property plots

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: FSP1 (tetrapod ancestor; L323A mutant) bound to FAD and NAD⁺ and compound 2



- Molecule 1: FSP1 (tetrapod ancestor; L323A mutant) bound to FAD and NAD⁺ and compound 2



4 Data and refinement statistics

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α , β , γ	44.45Å 78.06Å 115.08Å 90.00° 98.93° 90.00°	Depositor
Resolution (Å)	64.43 – 2.40 64.43 – 2.40	Depositor EDS
% Data completeness (in resolution range)	95.8 (64.43-2.40) 95.8 (64.43-2.40)	Depositor EDS
R_{merge}	0.15	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.81 (at 2.40Å)	Xtriage
Refinement program	REFMAC 5.8.0405	Depositor
R, R_{free}	0.216 , 0.279 0.225 , 0.280	Depositor DCC
R_{free} test set	1546 reflections (5.05%)	wwPDB-VP
Wilson B-factor (Å ²)	29.8	Xtriage
Anisotropy	0.092	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.35 , 45.1	EDS
L-test for twinning ²	$\langle L \rangle = 0.47$, $\langle L^2 \rangle = 0.30$	Xtriage
Estimated twinning fraction	0.087 for h,-k,-h-l	Xtriage
F_o, F_c correlation	0.91	EDS
Total number of atoms	5923	wwPDB-VP
Average B, all atoms (Å ²)	32.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 8.22% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical 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: NAD, A1I9W, FAD, CA

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	0.45	0/2806	0.74	0/3789
1	B	0.41	0/2823	0.70	0/3812
All	All	0.43	0/5629	0.72	0/7601

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	B	0	2

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	B	183	ARG	Sidechain
1	B	328	ARG	Sidechain

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	2759	0	2789	44	0
1	B	2775	0	2800	33	0
2	A	53	0	31	1	0
2	B	53	0	31	1	0
3	A	44	0	26	1	0
3	B	44	0	26	1	0
4	A	33	0	0	0	0
5	A	1	0	0	0	0
6	A	103	0	0	1	2
6	B	58	0	0	1	3
All	All	5923	0	5703	76	4

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 7.

All (76) 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:110:PRO:HG3	1:A:129:MET:CE	1.77	1.13
1:A:198:LEU:HD11	1:A:228:ILE:CD1	1.83	1.08
1:A:198:LEU:HD11	1:A:228:ILE:HD11	1.13	1.07
1:A:110:PRO:HG3	1:A:129:MET:HE1	0.99	0.97
1:A:110:PRO:CG	1:A:129:MET:HE1	1.96	0.94
1:A:346:ARG:HH21	1:A:351:ARG:NH2	1.66	0.92
1:A:346:ARG:NH2	1:A:351:ARG:HH22	1.69	0.90
1:A:346:ARG:HH21	1:A:351:ARG:HH22	0.89	0.85
1:A:346:ARG:HE	1:A:351:ARG:NH2	1.76	0.83
1:A:198:LEU:CD1	1:A:228:ILE:HD11	2.06	0.81
1:B:224:LYS:NZ	6:B:501:HOH:O	2.12	0.81
1:B:50:SER:HB2	1:B:129:MET:HG2	1.66	0.77
1:A:256:GLU:HB2	6:A:545:HOH:O	1.85	0.77
1:A:198:LEU:CD1	1:A:228:ILE:CD1	2.65	0.75
1:B:266:LEU:HD12	1:B:298:VAL:HB	1.75	0.69
1:A:346:ARG:NH2	1:A:351:ARG:NH2	2.33	0.68
1:B:163:GLU:OE2	1:B:165:THR:OG1	2.10	0.68
1:A:346:ARG:NE	1:A:351:ARG:NH2	2.40	0.68
1:A:315:TYR:O	1:A:317:PRO:HD3	1.98	0.64
1:A:145:GLY:HA2	1:A:174:ALA:HA	1.84	0.60
1:B:329:ASN:HA	1:B:346:ARG:HD2	1.86	0.58
1:A:4:ASP:CG	1:A:5:SER:H	2.07	0.57
1:B:266:LEU:HD11	1:B:295:HIS:HA	1.85	0.57
1:A:110:PRO:CG	1:A:129:MET:CE	2.67	0.57

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:265:HIS:HB3	1:A:302:ASN:ND2	2.19	0.57
1:B:50:SER:CB	1:B:129:MET:HG2	2.36	0.56
1:B:190:LEU:HD21	1:B:326:MET:HE1	1.87	0.56
1:A:109:PHE:CG	1:A:110:PRO:HA	2.41	0.55
1:A:136:ALA:HB2	1:A:214:VAL:HG22	1.89	0.55
1:B:143:GLY:O	1:B:148:GLY:HA3	2.08	0.54
1:A:346:ARG:CZ	1:A:351:ARG:NH2	2.71	0.53
1:A:143:GLY:O	1:A:166:LEU:HD11	2.07	0.53
1:B:117:VAL:HG21	1:B:125:MET:HE1	1.91	0.53
1:B:235:CYS:SG	1:B:237:THR:OG1	2.66	0.52
1:A:176:VAL:HA	1:A:183:ARG:NH1	2.24	0.52
1:B:346:ARG:O	1:B:351:ARG:HG2	2.10	0.52
1:A:9:VAL:HG11	1:A:86:VAL:HG21	1.90	0.52
1:B:331:GLY:O	1:B:342:ARG:HA	2.10	0.51
1:B:146[B]:SER:OG	3:B:402:NAD:H2D	2.11	0.50
1:B:35:ASP:OD1	2:B:401:FAD:H1B	2.11	0.50
1:B:138:ARG:HG2	1:B:163:GLU:HB3	1.94	0.50
1:A:150:GLU:OE1	1:A:325:SER:OG	2.30	0.50
1:B:235:CYS:HG	1:B:237:THR:HG1	1.57	0.49
1:A:346:ARG:NE	1:A:351:ARG:HH21	2.10	0.48
1:A:116:PRO:HB2	1:B:120:GLU:HG3	1.95	0.48
1:B:150:GLU:HG2	1:B:325:SER:O	2.13	0.48
1:A:266:LEU:HD12	1:A:298:VAL:HB	1.96	0.48
1:B:4:ASP:N	1:B:4:ASP:OD1	2.47	0.48
1:B:302:ASN:OD1	1:B:313:LYS:N	2.40	0.48
1:A:198:LEU:CD1	1:A:228:ILE:HD12	2.42	0.47
1:B:109:PHE:CG	1:B:110:PRO:HA	2.49	0.47
1:A:8:VAL:O	1:A:31:PHE:HA	2.14	0.47
1:A:287:LYS:O	3:A:402:NAD:H1D	2.15	0.47
1:B:190:LEU:CD2	1:B:326:MET:HE1	2.45	0.47
1:A:41:HIS:CD2	1:A:60:PHE:CE1	3.03	0.46
1:A:182:VAL:O	1:A:186:VAL:HG23	2.16	0.46
1:A:218:MET:O	1:A:219:LYS:HG3	2.15	0.46
1:A:57:LYS:HE2	1:A:367:PRO:HB2	1.97	0.46
1:B:282:ASP:OD1	1:B:284:LYS:NZ	2.47	0.46
1:A:110:PRO:HB3	1:A:129:MET:SD	2.57	0.45
1:B:301:THR:HG21	1:B:313:LYS:HD2	1.99	0.44
1:B:9:VAL:HG11	1:B:86:VAL:HG21	2.00	0.44
1:B:45:ALA:HA	1:B:353:LEU:HD13	2.00	0.43
1:A:329:ASN:O	1:A:346:ARG:HD2	2.19	0.42
1:B:168:HIS:CD2	1:B:173:LEU:HD23	2.55	0.42

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:210:THR:CG2	1:A:213:GLN:HG3	2.50	0.41
1:A:346:ARG:HE	1:A:351:ARG:HH21	1.60	0.41
1:B:109:PHE:CE1	1:B:110:PRO:HB3	2.55	0.41
1:B:138:ARG:HA	1:B:163:GLU:HB3	2.02	0.41
1:B:138:ARG:HG2	1:B:163:GLU:CB	2.50	0.41
1:A:75:LYS:HE3	1:A:89:GLU:OE1	2.21	0.41
1:B:265:HIS:O	1:B:266:LEU:HB2	2.20	0.41
1:A:288:MET:HA	2:A:401:FAD:H1'2	2.03	0.41
1:A:266:LEU:CD1	1:A:295:HIS:HA	2.51	0.41
1:B:265:HIS:CE1	1:B:312:LEU:HB2	2.56	0.41
1:A:104:GLY:HA2	1:A:279:ASP:HB2	2.04	0.40

All (4) symmetry-related close contacts are listed below. The label for Atom-2 includes the symmetry operator and encoded unit-cell translations to be applied.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
6:B:523:HOH:O	6:B:544:HOH:O[2_555]	1.56	0.64
6:B:521:HOH:O	6:B:539:HOH:O[2_645]	1.81	0.39
6:A:578:HOH:O	6:A:595:HOH:O[2_444]	1.84	0.36
6:A:556:HOH:O	6:B:542:HOH:O[1_455]	2.08	0.12

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	362/368 (98%)	342 (94%)	20 (6%)	0	100	100
1	B	364/368 (99%)	348 (96%)	15 (4%)	1 (0%)	37	51
All	All	726/736 (99%)	690 (95%)	35 (5%)	1 (0%)	48	65

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	B	251	GLY

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	297/301 (99%)	297 (100%)	0	100	100
1	B	299/301 (99%)	297 (99%)	2 (1%)	81	91
All	All	596/602 (99%)	594 (100%)	2 (0%)	91	96

All (2) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	4	ASP
1	B	51	VAL

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (3) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	168	HIS
1	A	273	ASN
1	A	297	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 [i](#)

There are no oligosaccharides in this entry.

5.6 Ligand geometry [i](#)

Of 6 ligands modelled in this entry, 1 is 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	NAD	B	402	-	42,48,48	0.69	2 (4%)	50,73,73	0.73	1 (2%)
4	A1I9W	A	403	-	34,37,37	1.49	3 (8%)	41,51,51	2.46	5 (12%)
2	FAD	A	401	-	53,58,58	0.73	0	68,89,89	0.70	1 (1%)
3	NAD	A	402	-	42,48,48	0.68	2 (4%)	50,73,73	0.72	1 (2%)
2	FAD	B	401	-	53,58,58	0.73	0	68,89,89	0.73	2 (2%)

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	NAD	B	402	-	-	2/26/62/62	0/5/5/5
4	A1I9W	A	403	-	-	2/15/38/38	0/5/5/5
2	FAD	A	401	-	-	1/30/50/50	0/6/6/6
3	NAD	A	402	-	-	1/26/62/62	0/5/5/5
2	FAD	B	401	-	-	2/30/50/50	0/6/6/6

All (7) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
4	A	403	A1I9W	C27-C26	-5.43	1.33	1.52
4	A	403	A1I9W	C22-C23	-5.20	1.34	1.52
3	A	402	NAD	C2N-N1N	2.32	1.37	1.35
3	B	402	NAD	C2N-N1N	2.32	1.37	1.35

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Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
4	A	403	A1I9W	C23-C24	-2.11	1.39	1.49
3	B	402	NAD	C8A-N7A	-2.04	1.31	1.34
3	A	402	NAD	C8A-N7A	-2.02	1.31	1.34

All (10) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
4	A	403	A1I9W	C26-C27-C21	8.97	128.69	113.96
4	A	403	A1I9W	C23-C22-C21	8.34	127.66	113.96
4	A	403	A1I9W	C27-C21-C22	-6.28	96.17	113.71
4	A	403	A1I9W	C27-C26-C25	3.91	125.37	116.36
4	A	403	A1I9W	C22-C23-C24	3.82	125.15	116.36
2	A	401	FAD	C5A-C6A-N6A	2.38	123.97	120.35
2	B	401	FAD	C5A-C6A-N6A	2.35	123.92	120.35
3	B	402	NAD	C5A-C6A-N6A	2.24	123.76	120.35
3	A	402	NAD	C5A-C6A-N6A	2.22	123.73	120.35
2	B	401	FAD	C4-N3-C2	-2.01	121.92	125.64

There are no chirality outliers.

All (8) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
4	A	403	A1I9W	O1-C1-N1-C2
2	B	401	FAD	O4B-C4B-C5B-O5B
4	A	403	A1I9W	C21-C1-N1-C2
3	B	402	NAD	PA-O3-PN-O5D
2	B	401	FAD	C3B-C4B-C5B-O5B
3	A	402	NAD	O4B-C4B-C5B-O5B
3	B	402	NAD	O4B-C4B-C5B-O5B
2	A	401	FAD	O4B-C4B-C5B-O5B

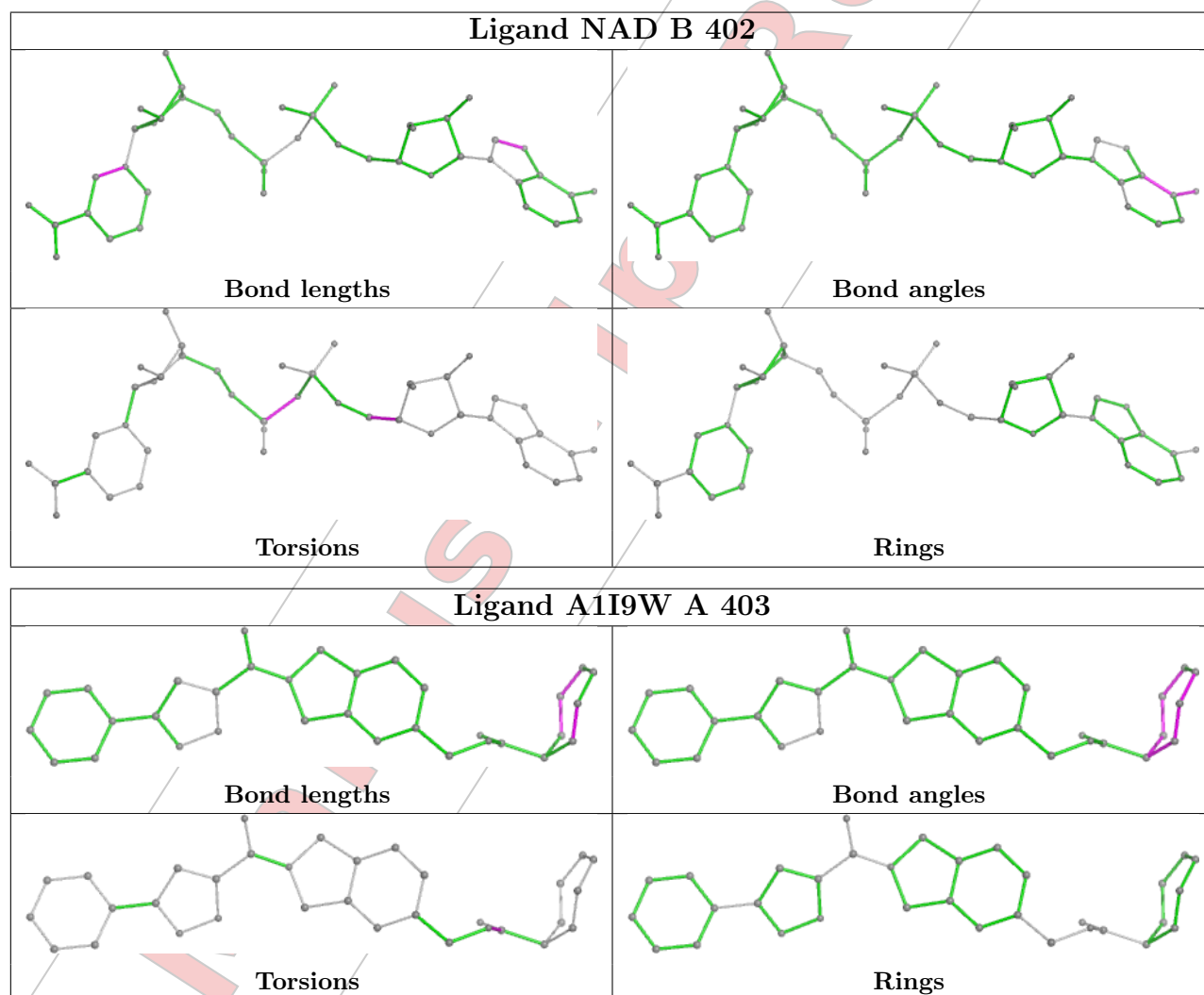
There are no ring outliers.

4 monomers are involved in 4 short contacts:

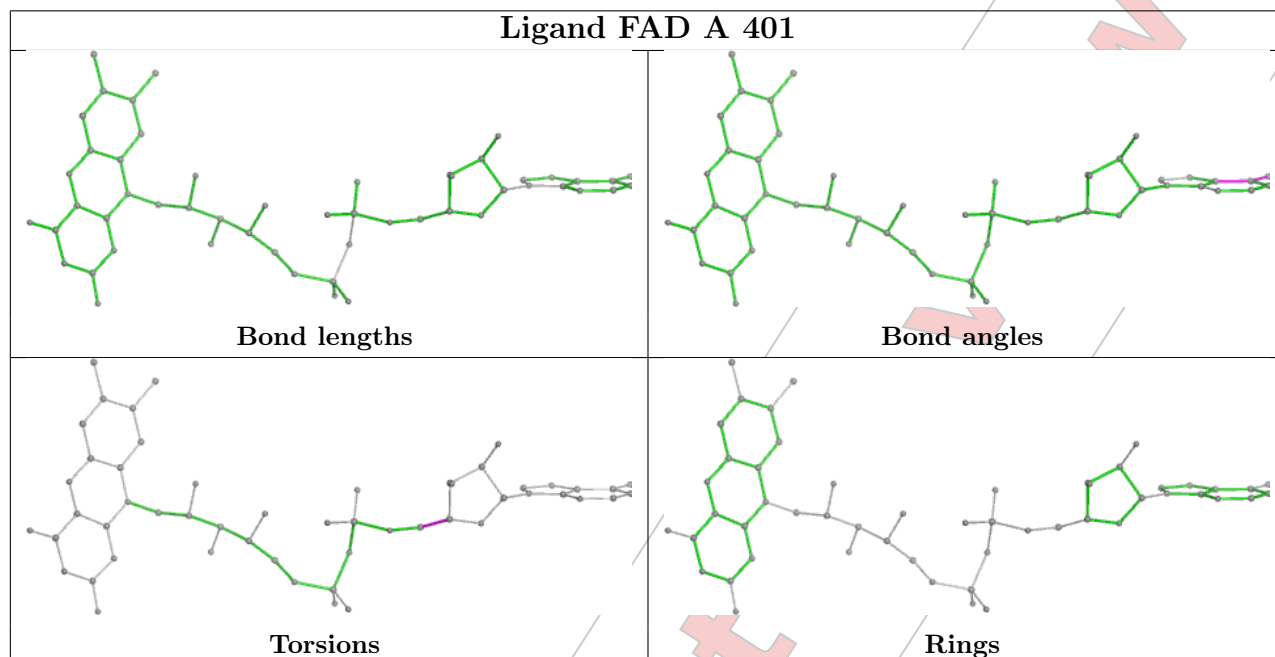
Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	B	402	NAD	1	0
2	A	401	FAD	1	0
3	A	402	NAD	1	0
2	B	401	FAD	1	0

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths,

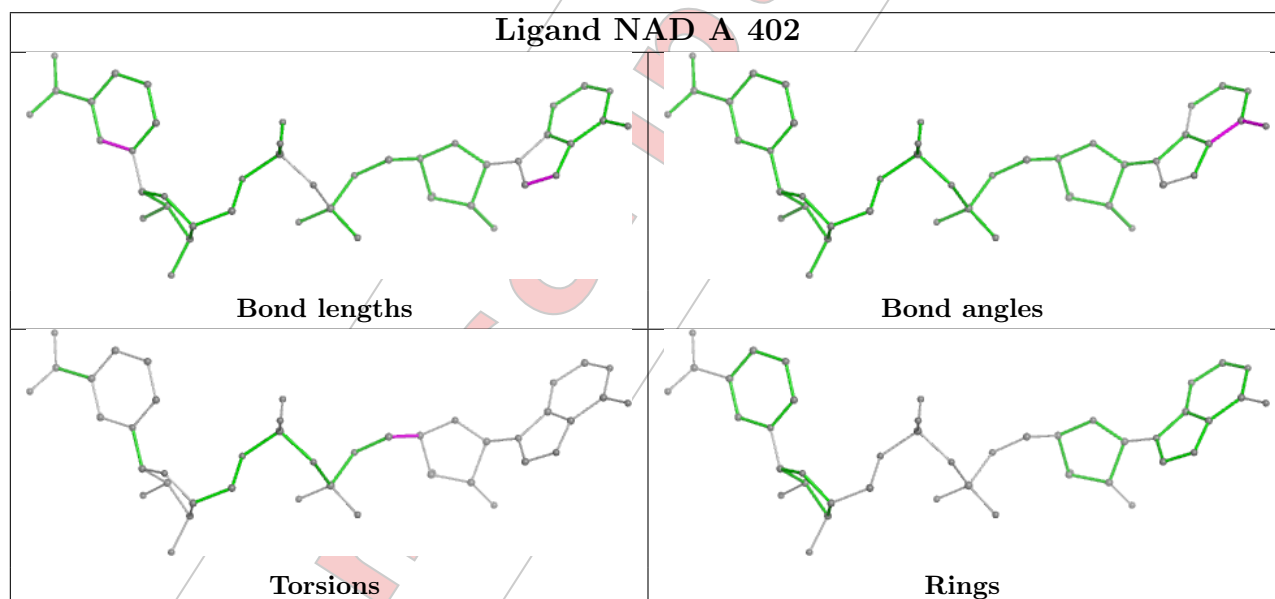
bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less than 5% of the Mogul distribution of torsion angles is within 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.

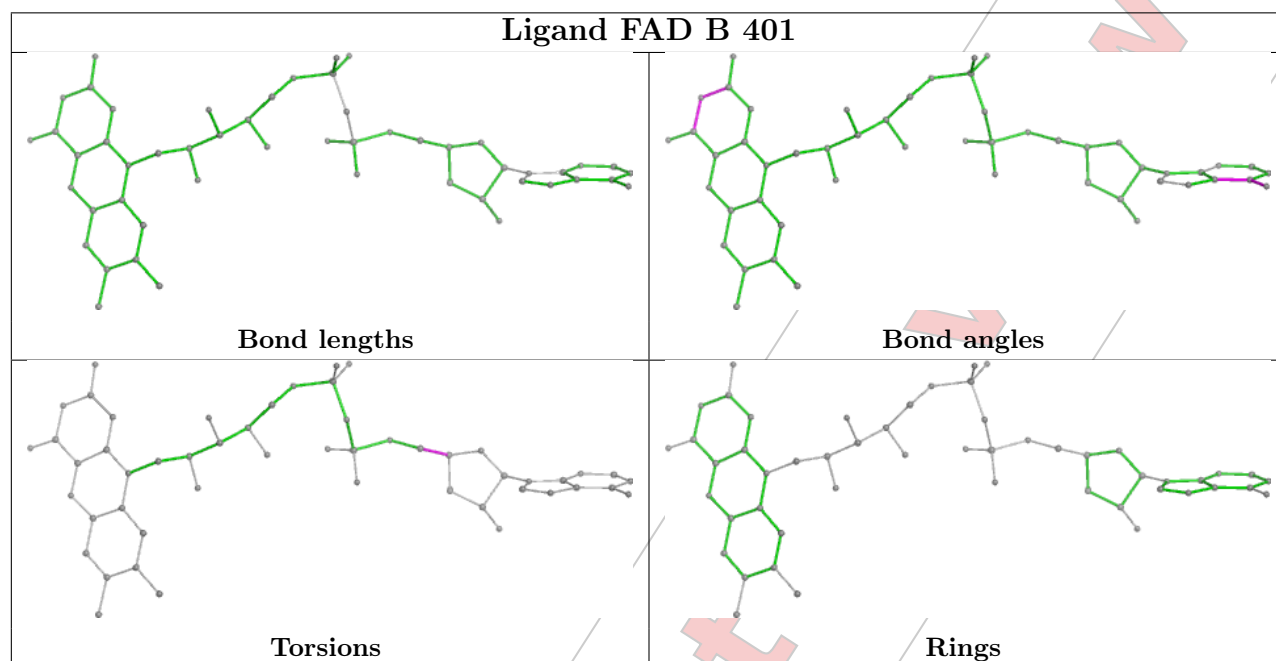


Ligand FAD A 401



Ligand NAD A 402





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, 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	364/368 (98%)	-0.12	5 (1%) 73 70	14, 26, 50, 78	0
1	B	365/368 (99%)	0.06	10 (2%) 56 53	10, 32, 56, 84	1 (0%)
All	All	729/736 (99%)	-0.03	15 (2%) 63 60	10, 30, 54, 84	1 (0%)

All (15) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	317	PRO	5.4
1	B	116	PRO	4.9
1	A	318	GLY	4.7
1	B	82	GLU	3.0
1	A	115	GLN	2.9
1	A	129	MET	2.6
1	B	202	ARG	2.4
1	A	196	GLN	2.4
1	B	115	GLN	2.4
1	B	207	GLN	2.4
1	B	310	LYS	2.2
1	B	317	PRO	2.2
1	B	4	ASP	2.2
1	B	309	ASN	2.1
1	B	171	ILE	2.1

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](#)

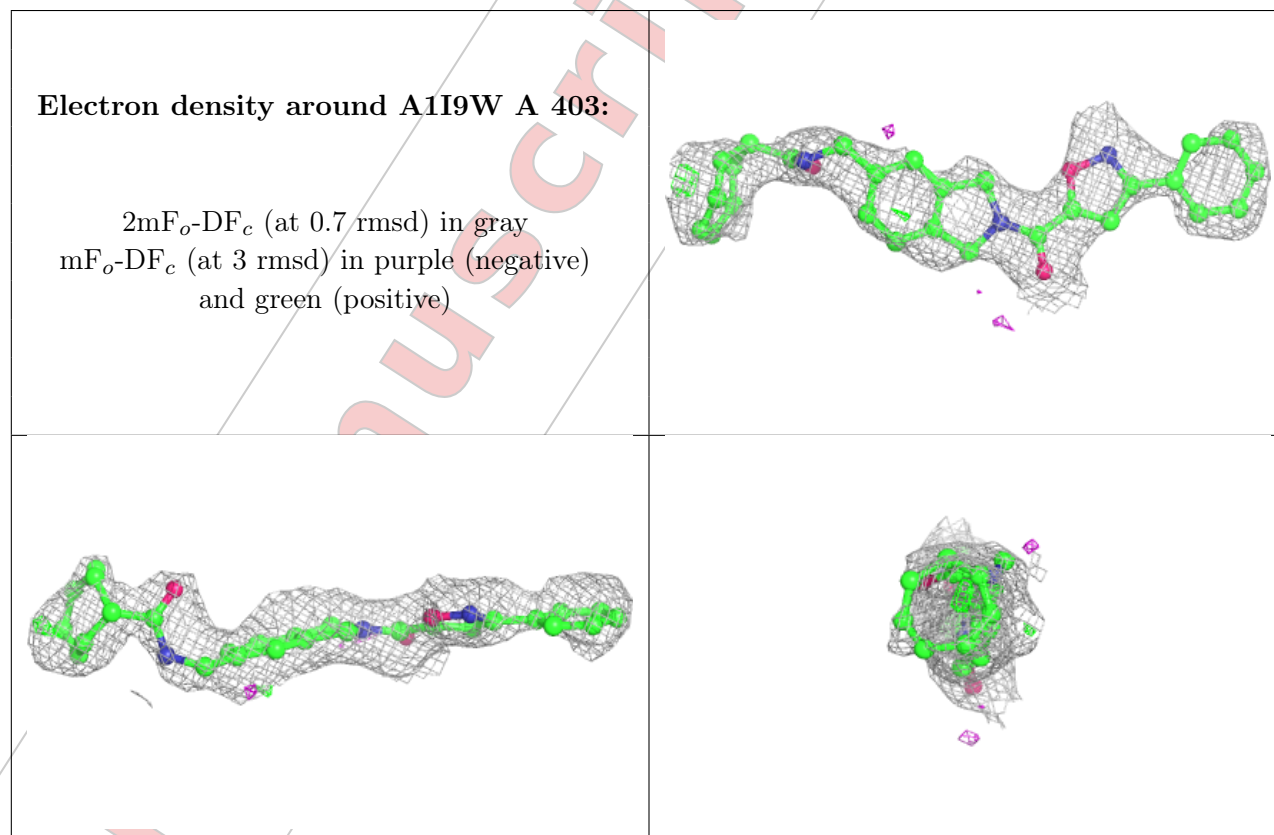
There are no monosaccharides in this entry.

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, 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.

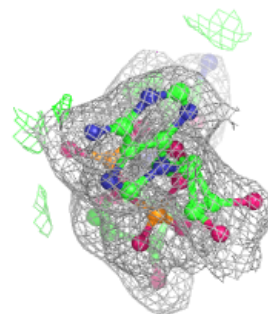
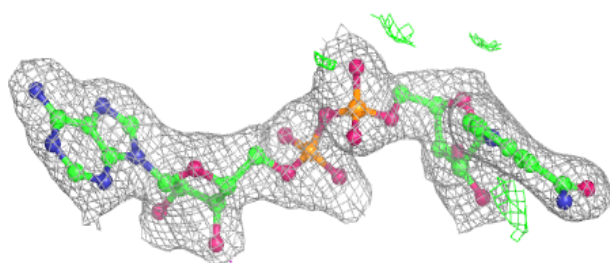
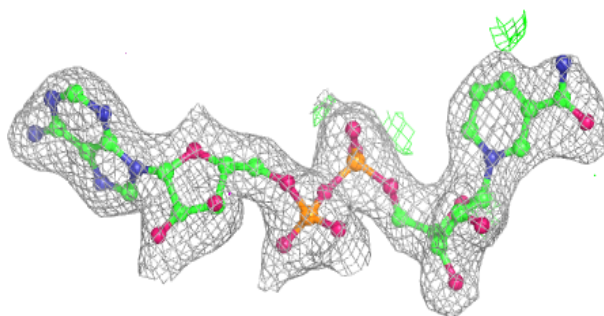
Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å ²)	Q<0.9
4	A1I9W	A	403	33/33	0.87	0.15	34,50,58,62	0
3	NAD	A	402	44/44	0.95	0.07	19,24,27,29	0
3	NAD	B	402	44/44	0.95	0.07	17,23,30,34	0
2	FAD	B	401	53/53	0.95	0.08	16,23,29,35	0
2	FAD	A	401	53/53	0.96	0.07	16,19,24,27	0
5	CA	A	404	1/1	0.97	0.09	53,53,53,53	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.



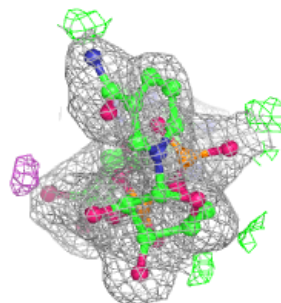
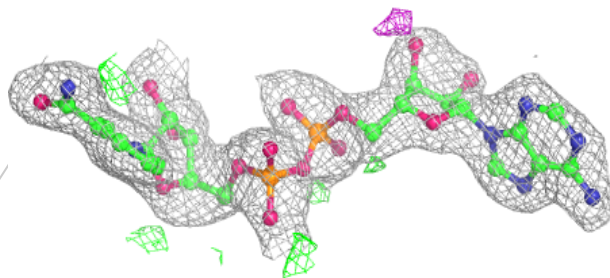
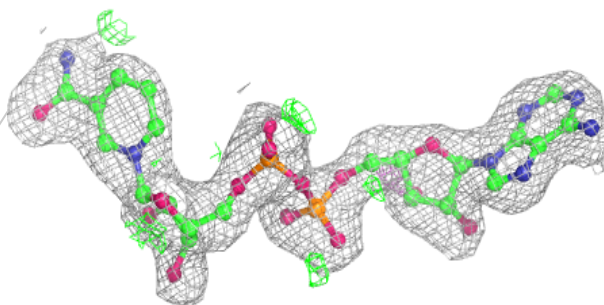
Electron density around NAD A 402:

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



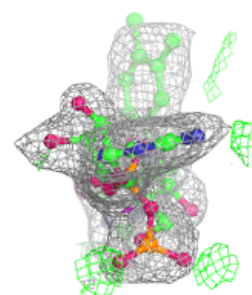
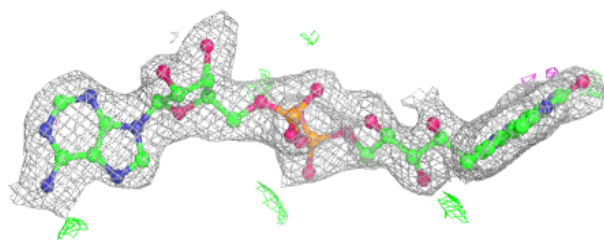
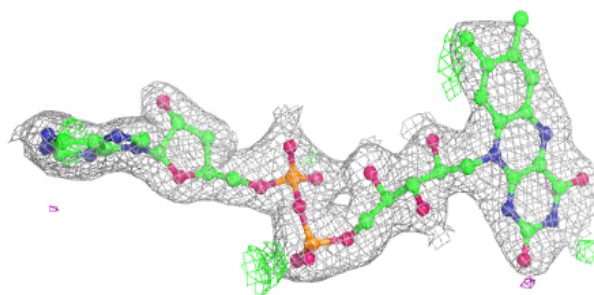
Electron density around NAD B 402:

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



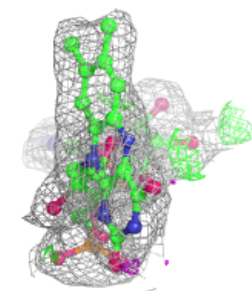
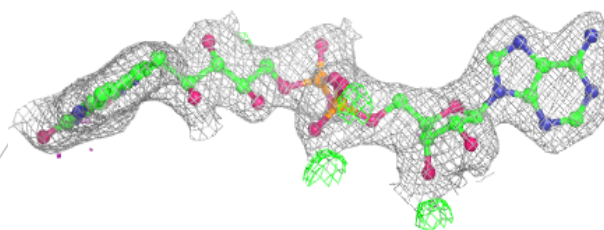
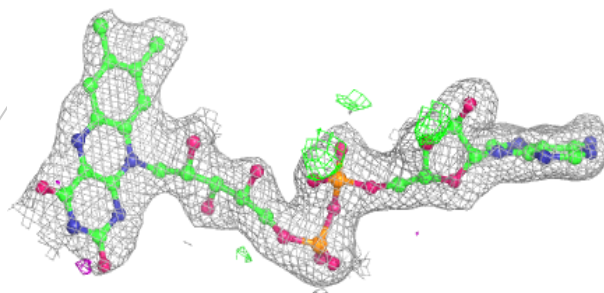
Electron density around FAD B 401:

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



Electron density around FAD A 401:

$2mF_o-DF_c$ (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.

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