WORLDWIDE PDB PROTEIN DATA BANK

Full wwPDB EM Validation Report (i)

Jul 14, 2024 – 12:06 AM JST

PDB ID	:	8JO3
EMDB ID	:	EMD-36454
Title	:	Cryo-EM structure of a Legionella effector complexed with actin and AMP
Authors	:	Zhou, X.T.; Wang, X.F.; Tan, J.X.; Zhu, Y.Q.
Deposited on	:	2023-06-07
Resolution	:	2.66 Å(reported)
Based on initial model	:	.

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

|		
EMDB validation analysis	:	FAILED
Mogul	:	1.8.5 (274361), CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	FAILED
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.37.1

1 Overall quality at a glance ⓘ

The following experimental techniques were used to determine the structure:
ELECTRON MICROSCOPY

The reported resolution of this entry is 2.66 Å.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.

Metric	Percentile Ranks	Value
Clashscore		9
Ramachandran outliers		0
Sidechain outliers		3.8%

Worse   *Better*

▮ Percentile relative to all structures
◻ Percentile relative to all EM structures
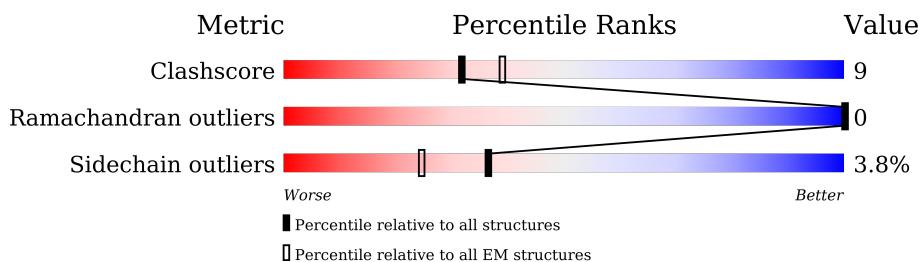
Metric	Whole archive (#Entries)	EM structures (#Entries)
Clashscore	158937	4297
Ramachandran outliers	154571	4023
Sidechain outliers	154315	3826

The table below summarises the geometric issues observed across the polymeric chains and their fit to the map. The red, orange, yellow and green segments of the bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria respectively. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5%

Mol	Chain	Length	Quality of chain
1	A	436	58% 21% • 20%
2	B	377	71% 24% • 5%

2 Entry composition ⓘ

There are 5 unique types of molecules in this entry. The entry contains 5715 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, 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 Substrate of the Dot/Icm secretion system.

| Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	350	Total 2851	C 1817	N 474	O 552	S 8	0	0

There are 11 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	426	SER	-	expression tag	UNP A0A2H5FI52
A	427	SER	-	expression tag	UNP A0A2H5FI52
A	428	GLY	-	expression tag	UNP A0A2H5FI52
A	429	TRP	-	expression tag	UNP A0A2H5FI52
A	430	SER	-	expression tag	UNP A0A2H5FI52
A	431	HIS	-	expression tag	UNP A0A2H5FI52
A	432	PRO	-	expression tag	UNP A0A2H5FI52
A	433	GLN	-	expression tag	UNP A0A2H5FI52
A	434	PHE	-	expression tag	UNP A0A2H5FI52
A	435	GLU	-	expression tag	UNP A0A2H5FI52
A	436	LYS	-	expression tag	UNP A0A2H5FI52

- Molecule 2 is a protein called Actin, alpha skeletal muscle.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	B	359	Total 2809	C 1782	N 469	O 539	S 19	0	0

- Molecule 3 is ADENOSINE MONOPHOSPHATE (three-letter code: AMP) (formula: $C_{10}H_{14}N_5O_7P$) (labeled as "Ligand of Interest" by depositor).

WORLDWIDE
wwPDB
PROTEIN DATA BANK

AMP

Mol	Chain	Residues	Atoms					AltConf
3	A	1	Total	C	N	O	P	0
			23	10	5	7	1	

- Molecule 4 is CALCIUM ION (three-letter code: CA) (formula: Ca).

Mol	Chain	Residues	Atoms		AltConf
4	B	1	Total	Ca	0
			1	1	

- Molecule 5 is ADENOSINE-5'-TRIPHOSPHATE (three-letter code: ATP) (formula: $C_{10}H_{16}N_5O_{13}P_3$).

ATP
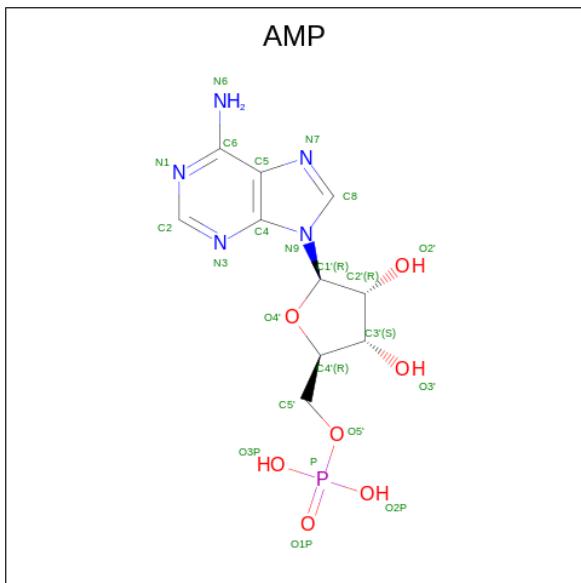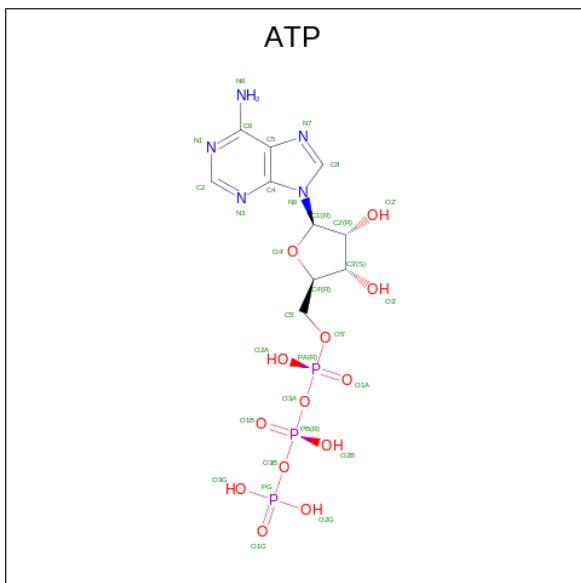
| Mol	Chain	Residues	Atoms					AltConf
			Total	C	N	O	P	
5	B	1	31	10	5	13	3	0

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. 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. 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: Substrate of the Dot/Icm secretion system

Chain A:
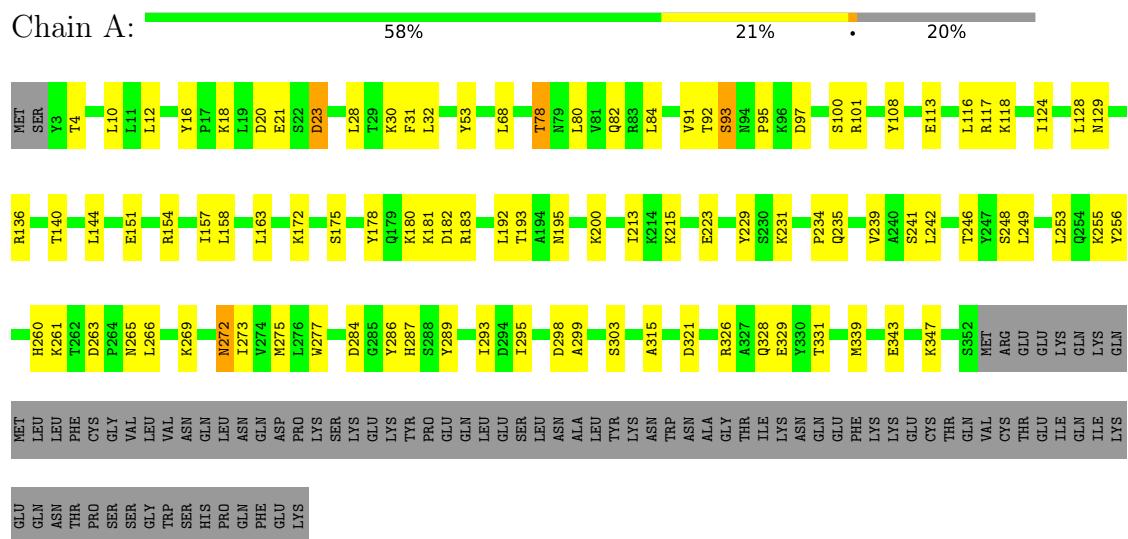
• Molecule 2: Actin, alpha skeletal muscle

Chain B:
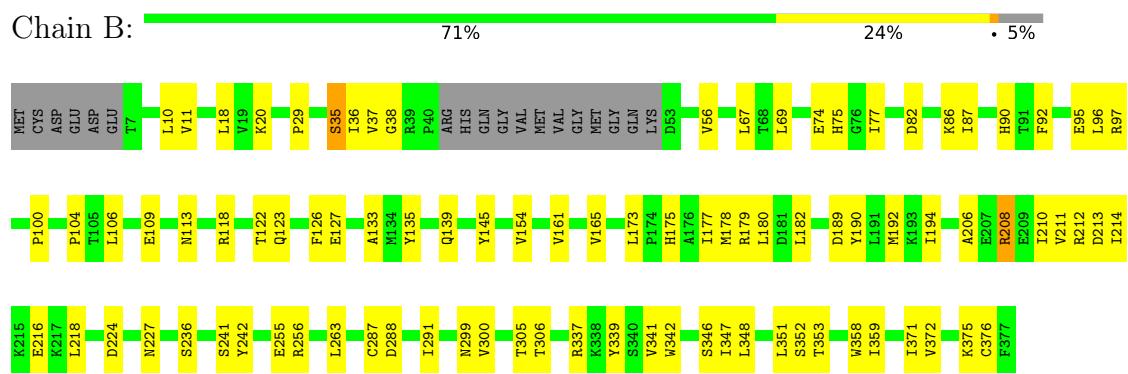
4 Experimental information ⓘ

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C1	Depositor
Number of particles used	868128	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose $(e^-/\text{Å}^2)$	50	Depositor
Minimum defocus (nm)	800	Depositor
Maximum defocus (nm)	1600	Depositor
Magnification	Not provided	
Image detector	FEI FALCON IV (4k x 4k)	Depositor

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: AMP, CA, ATP

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	0.26	0/2902	0.43	0/3917
2	B	0.27	0/2870	0.47	0/3890
All	All	0.26	0/5772	0.45	0/7807

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	2851	0	2867	58	0
2	B	2809	0	2776	54	0
3	A	23	0	12	3	0
4	B	1	0	0	0	0
5	B	31	0	12	1	0
All	All	5715	0	5667	108	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 9.

All (108) 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)
2:B:37:VAL:HG22	2:B:56:VAL:HG22	1.71	0.72
2:B:133:ALA:HB1	2:B:358:TRP:HB3	1.71	0.72
2:B:211:VAL:HA	2:B:214:ILE:HD12	1.72	0.71
1:A:158:LEU:O	3:A:501:AMP:N6	2.25	0.68
1:A:30:LYS:HD3	1:A:328:GLN:HB3	1.76	0.67
1:A:117:ARG:NH2	1:A:151:GLU:OE1	2.29	0.66
1:A:23:ASP:OD2	1:A:331:THR:HG23	1.94	0.66
2:B:109:GLU:OE2	2:B:118:ARG:NH1	2.29	0.66
2:B:306:THR:O	2:B:337:ARG:NH1	2.28	0.65
1:A:92:THR:HG22	1:A:93:SER:H	1.62	0.64
1:A:95:PRO:HG3	1:A:234:PRO:HA	1.81	0.63
1:A:347:LYS:HD3	2:B:347:ILE:HG23	1.81	0.62
2:B:224:ASP:HB3	2:B:227:ASN:HB2	1.83	0.61
1:A:246:THR:HB	1:A:295:ILE:HG21	1.83	0.60
1:A:154:ARG:NH2	1:A:284:ASP:OD1	2.34	0.60
1:A:97:ASP:OD1	1:A:200:LYS:NZ	2.28	0.59
1:A:195:ASN:ND2	1:A:200:LYS:O	2.34	0.59
2:B:36:ILE:HD12	2:B:69:LEU:HD13	1.84	0.59
1:A:129:ASN:ND2	1:A:144:LEU:O	2.37	0.58
2:B:371:ILE:HG13	2:B:375:LYS:HG3	1.86	0.58
2:B:90:HIS:NE2	2:B:95:GLU:OE2	2.36	0.58
1:A:287:HIS:NE2	3:A:501:AMP:O1P	2.37	0.57
2:B:75:HIS:O	2:B:179:ARG:NH2	2.38	0.57
1:A:124:ILE:HG12	1:A:269:LYS:HG2	1.86	0.56
1:A:183:ARG:HB2	1:A:241:SER:HB2	1.86	0.56
1:A:108:TYR:OH	1:A:315:ALA:O	2.20	0.56
1:A:242:LEU:HB3	1:A:295:ILE:HD11	1.87	0.56
1:A:183:ARG:HH12	3:A:501:AMP:H3'	1.70	0.55
2:B:122:THR:HG21	2:B:372:VAL:HB	1.89	0.55
2:B:190:TYR:O	2:B:194:ILE:HG13	2.06	0.54
2:B:118:ARG:NH2	2:B:376:CYS:O	2.33	0.54
1:A:158:LEU:HD22	1:A:286:TYR:HE2	1.73	0.54
1:A:234:PRO:HD2	1:A:235:GLN:NE2	2.23	0.53
1:A:343:GLU:OE1	2:B:353:THR:OG1	2.20	0.53
2:B:77:ILE:HG12	2:B:179:ARG:HH22	1.74	0.53
1:A:12:LEU:HG	1:A:339:MET:HG3	1.91	0.52
2:B:35:SER:O	2:B:35:SER:OG	2.27	0.52
1:A:78:THR:O	1:A:82:GLN:NE2	2.43	0.52
2:B:11:VAL:HG21	2:B:346:SER:HA	1.92	0.52
1:A:343:GLU:OE2	2:B:352:SER:OG	2.28	0.52
2:B:20:LYS:HD3	2:B:339:TYR:HD1	1.75	0.50
1:A:113:GLU:O	1:A:117:ARG:HG3	2.11	0.50

Continued on next page...

Continued from previous page...

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:289:TYR:CZ	1:A:293:ILE:HG13	2.46	0.50
2:B:154:VAL:HG22	2:B:300:VAL:HB	1.93	0.50
2:B:18:LEU:O	2:B:20:LYS:NZ	2.43	0.50
2:B:190:TYR:CE2	2:B:194:ILE:HD11	2.46	0.50
2:B:212:ARG:NH1	2:B:216:GLU:OE2	2.45	0.50
1:A:32:LEU:HD13	1:A:68:LEU:HD23	1.94	0.50
2:B:113:ASN:HD22	2:B:118:ARG:HG3	1.76	0.49
2:B:104:PRO:HB3	2:B:133:ALA:HB3	1.92	0.49
1:A:182:ASP:OD2	1:A:231:LYS:NZ	2.46	0.49
1:A:253:LEU:HD21	1:A:273:ILE:HG21	1.94	0.49
1:A:249:LEU:HD22	1:A:277:TRP:CD2	2.48	0.49
2:B:189:ASP:OD1	2:B:208:ARG:NH1	2.46	0.49
1:A:347:LYS:HD2	2:B:351:LEU:HD21	1.94	0.48
1:A:157:ILE:HG23	1:A:277:TRP:CE3	2.48	0.48
2:B:180:LEU:HG	2:B:182:LEU:HB3	1.95	0.48
2:B:182:LEU:HD21	2:B:263:LEU:HA	1.95	0.48
1:A:128:LEU:HD13	1:A:273:ILE:HD13	1.96	0.47
1:A:20:ASP:H	1:A:23:ASP:HB2	1.79	0.47
1:A:192:LEU:HG	1:A:215:LYS:HE3	1.96	0.47
2:B:210:ILE:HD11	2:B:242:TYR:HE2	1.79	0.47
1:A:256:TYR:O	1:A:260:HIS:ND1	2.49	0.46
2:B:82:ASP:O	2:B:86:LYS:HG3	2.15	0.46
2:B:206:ALA:O	2:B:210:ILE:HG22	2.15	0.46
1:A:16:TYR:CZ	1:A:18:LYS:HB2	2.51	0.46
1:A:28:LEU:HA	1:A:28:LEU:HD23	1.68	0.46
1:A:116:LEU:HD23	1:A:272:ASN:HD21	1.80	0.46
1:A:136:ARG:NH1	1:A:140:THR:O	2.48	0.46
2:B:218:LEU:O	2:B:256:ARG:NH1	2.45	0.46
1:A:181:LYS:HE3	1:A:241:SER:HB3	1.98	0.46
2:B:139:GLN:HG2	2:B:341:VAL:HG11	1.98	0.45
2:B:10:LEU:HD22	2:B:96:LEU:HD13	1.98	0.45
1:A:80:LEU:O	1:A:84:LEU:HG	2.17	0.45
1:A:53:TYR:O	1:A:101:ARG:NH1	2.44	0.45
1:A:180:LYS:O	1:A:229:TYR:N	2.47	0.44
2:B:165:VAL:HG13	2:B:177:ILE:HG12	1.99	0.44
2:B:29:PRO:HG3	2:B:342:TRP:CG	2.53	0.44
2:B:118:ARG:O	2:B:122:THR:HG23	2.18	0.44
2:B:192:MET:HG2	2:B:211:VAL:HG21	2.00	0.44
2:B:38:GLY:HA3	2:B:67:LEU:HD13	1.99	0.44
1:A:263:ASP:HB3	1:A:266:LEU:HB2	2.00	0.43
1:A:113:GLU:OE2	1:A:117:ARG:NH1	2.50	0.43

Continued on next page...

Continued from previous page...

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
2:B:106:LEU:HD13	2:B:135:TYR:HB3	2.00	0.43
1:A:113:GLU:HA	1:A:116:LEU:HD12	2.00	0.43
2:B:255:GLU:OE1	2:B:255:GLU:N	2.36	0.43
1:A:10:LEU:HD22	1:A:31:PHE:CG	2.53	0.43
1:A:193:THR:O	1:A:213:ILE:HA	2.19	0.43
1:A:326:ARG:NH1	1:A:329:GLU:OE2	2.51	0.43
2:B:75:HIS:HA	2:B:161:VAL:HB	2.00	0.43
2:B:118:ARG:NH2	2:B:372:VAL:O	2.52	0.42
2:B:145:TYR:CD2	2:B:348:LEU:HD13	2.55	0.42
1:A:249:LEU:HD22	1:A:277:TRP:CG	2.55	0.42
1:A:118:LYS:HB3	1:A:118:LYS:HE3	1.71	0.42
2:B:56:VAL:HG13	2:B:87:ILE:HD13	2.02	0.42
2:B:287:CYS:HB3	2:B:291:ILE:HD11	2.02	0.42
1:A:21:GLU:HG2	1:A:91:VAL:HG23	2.02	0.41
1:A:20:ASP:OD1	1:A:20:ASP:N	2.53	0.41
2:B:216:GLU:HG2	5:B:402:ATP:C5	2.55	0.41
1:A:223:GLU:H	1:A:223:GLU:HG2	1.68	0.41
2:B:123:GLN:O	2:B:127:GLU:HG3	2.20	0.41
1:A:172:LYS:HE2	1:A:178:TYR:CZ	2.55	0.41
2:B:126:PHE:HZ	2:B:359:ILE:O	2.04	0.41
1:A:298:ASP:OD1	1:A:299:ALA:N	2.54	0.41
2:B:173:LEU:HB3	2:B:175:HIS:CE1	2.56	0.41
2:B:305:THR:O	2:B:305:THR:HG22	2.20	0.41
1:A:163:LEU:HB2	1:A:248:SER:HB3	2.03	0.40
2:B:92:PHE:HB3	2:B:100:PRO:HG3	2.04	0.40

There are no symmetry-related clashes.

5.3   Torsion angles ⓘ

5.3.1   Protein backbone ⓘ

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all EM entries.

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	348/436 (80%)	344 (99%)	4 (1%)	0	100	100

Continued on next page...

Continued from previous page...

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
2	B	355/377 (94%)	349 (98%)	6 (2%)	0	100	100
All	All	703/813 (86%)	693 (99%)	10 (1%)	0	100	100

There are no Ramachandran outliers to report.

5.3.2   Protein sidechains ⓘ

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all EM entries.

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	319/400 (80%)	305 (96%)	14 (4%)	28	43
2	B	305/320 (95%)	295 (97%)	10 (3%)	38	54
All	All	624/720 (87%)	600 (96%)	24 (4%)	36	49

All (24) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	4	THR
1	A	23	ASP
1	A	78	THR
1	A	93	SER
1	A	100	SER
1	A	175	SER
1	A	239	VAL
1	A	255	LYS
1	A	261	LYS
1	A	265	ASN
1	A	272	ASN
1	A	275	MET
1	A	303	SER
1	A	321	ASP
2	B	35	SER
2	B	74	GLU
2	B	97	ARG
2	B	178	MET

Continued on next page...

Continued from previous page...

Mol	Chain	Res	Type
2	B	208	ARG
2	B	213	ASP
2	B	236	SER
2	B	241	SER
2	B	288	ASP
2	B	299	ASN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (7) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	56	GLN
1	A	82	GLN
1	A	148	ASN
1	A	272	ASN
1	A	302	GLN
1	A	332	GLN
2	B	94	ASN

5.3.3   RNA ⓘ

There are no RNA molecules in this entry.

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

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

5.5   Carbohydrates ⓘ

There are no monosaccharides in this entry.

5.6   Ligand geometry ⓘ

Of 3 ligands modelled in this entry, 1 is monoatomic - leaving 2 for Mogul analysis.

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the

expected value. A bond length (or angle) with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\#|Z| > 2$	Counts	RMSZ	$\#|Z| > 2$
5	ATP	B	402	4	26,33,33	0.61	0	31,52,52	0.74	2 (6%)
3	AMP	A	501	-	22,25,25	0.87	1 (4%)	25,38,38	1.15	2 (8%)

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
5	ATP	B	402	4	-	5/18/38/38	0/3/3/3
3	AMP	A	501	-	-	0/6/26/26	0/3/3/3

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	A	501	AMP	C5-C4	2.39	1.47	1.40

All (4) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	A	501	AMP	N3-C2-N1	-3.12	123.80	128.68
5	B	402	ATP	C5-C6-N6	2.30	123.84	120.35
3	A	501	AMP	C4-C5-N7	-2.27	107.03	109.40
5	B	402	ATP	PB-O3B-PG	2.06	139.90	132.83

There are no chirality outliers.

All (5) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
5	B	402	ATP	PB-O3B-PG-O1G
5	B	402	ATP	PG-O3B-PB-O1B
5	B	402	ATP	PG-O3B-PB-O2B
5	B	402	ATP	PB-O3B-PG-O2G
5	B	402	ATP	PB-O3B-PG-O3G

There are no ring outliers.

2 monomers are involved in 4 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
5	B	402	ATP	1	0
3	A	501	AMP	3	0

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less then 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.

Ligand ATP B 402

Bond lengths

Bond angles

Torsions

Rings

Ligand AMP A 501

Bond lengths

Bond angles

Torsions

Rings
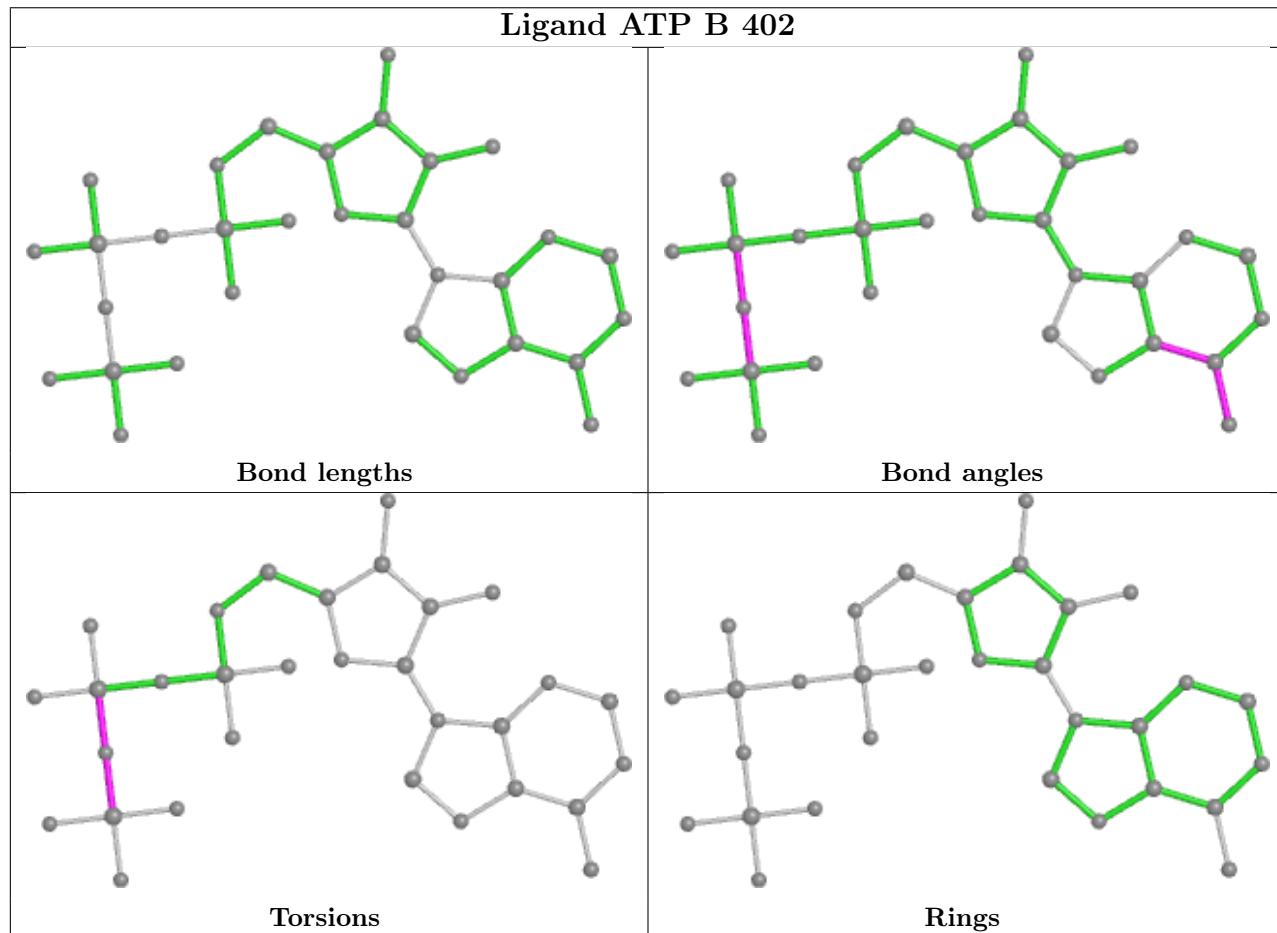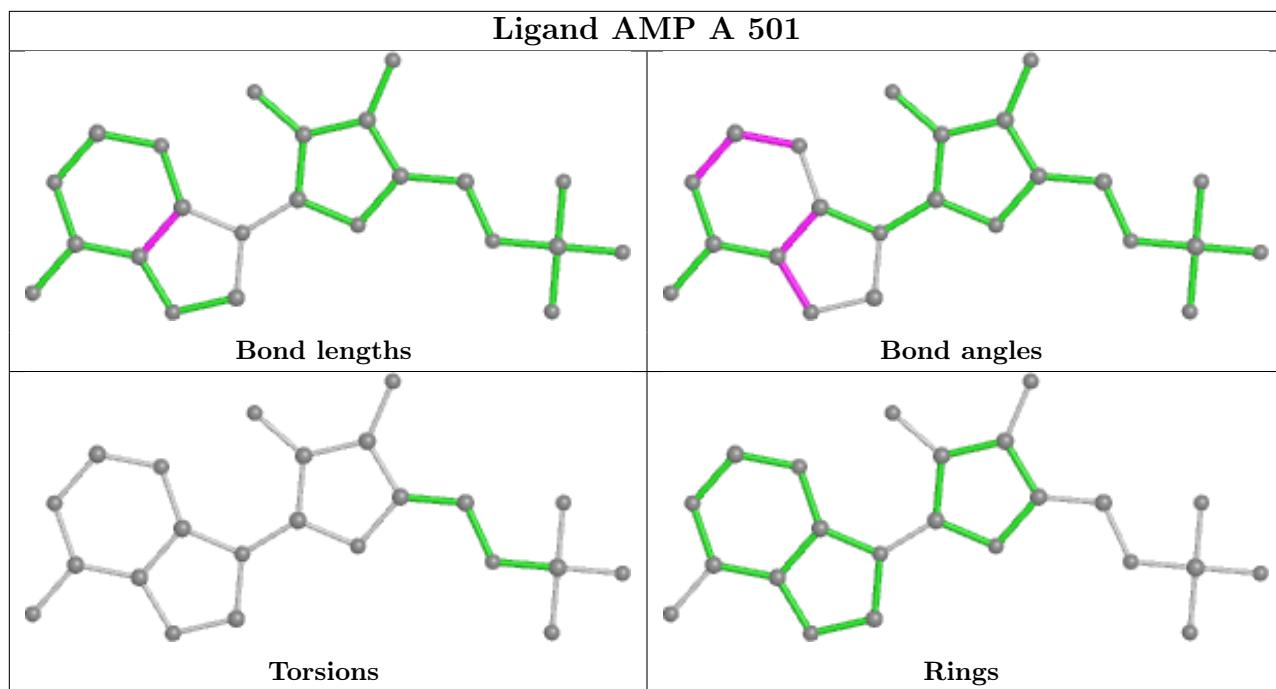
5.7 Other polymers ⓘ

There are no such residues in this entry.

5.8 Polymer linkage issues ⓘ

There are no chain breaks in this entry.