WORLDWIDE PDB PROTEIN DATA BANK

Full wwPDB EM Validation Report ⓘ

Mar 18, 2025 – 12:08 PM EDT

PDB ID	:	9NBP
Title	:	MicroED structure of the papain-E-64 complex from microcrystals mixed on-grid with microarrayed ligand
Authors	:	Vlahakis, N.; Rodriguez, J.A.
Deposited on	:	2025-02-14
Resolution	:	2.80 Å(reported)
Based on initial model	:	9PAP

This is a Full wwPDB EM Validation Report for a publicly released PDB/EMDB entry.

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
https://www.wwpdb.org/validation/2017/EMValidationReportHelp
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	:	2022.3.0, CSD as543be (2022)
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20231227.v01 (using entries in the PDB archive December 27th 2023)
CCP4	:	9.0.004 (Gargrove)
Density-Fitness	:	1.0.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.41.4

1 Overall quality at a glance ⓘ

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

The reported resolution of this entry is 2.80 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.
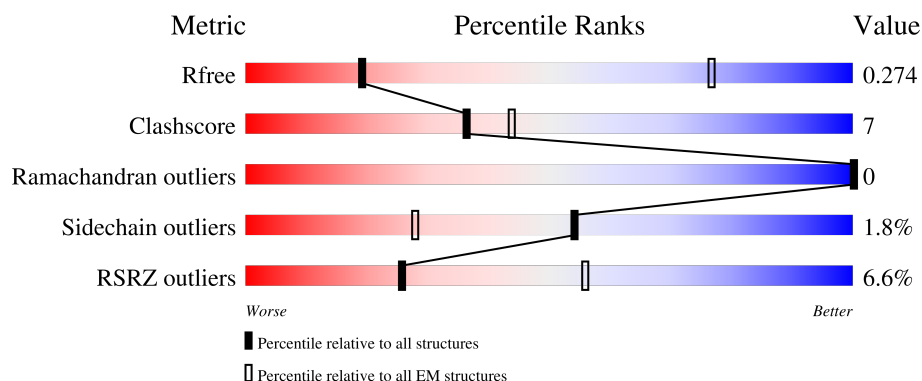
Metric	Whole archive (#Entries)	EM structures (#Entries)
R_{free}	164678	53
Clashscore	210492	15764
Ramachandran outliers	207382	16835
Sidechain outliers	206894	16415
RSRZ outliers	164674	54

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	212	7% 81% 19%

2 Entry composition ⓘ

There are 3 unique types of molecules in this entry. The entry contains 1717 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 Papain.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	212	Total 1682	C 1071	N 294	O 310	S 7	3	0

- Molecule 2 is N-[N-[1-HYDROXYCARBOXYETHYL-CARBONYL]LEUCYLAMINO-BUTYL]-GUANIDINE (three-letter code: E64) (formula: $C_{15}H_{30}N_5O_5$) (labeled as "Ligand of Interest" by depositor).
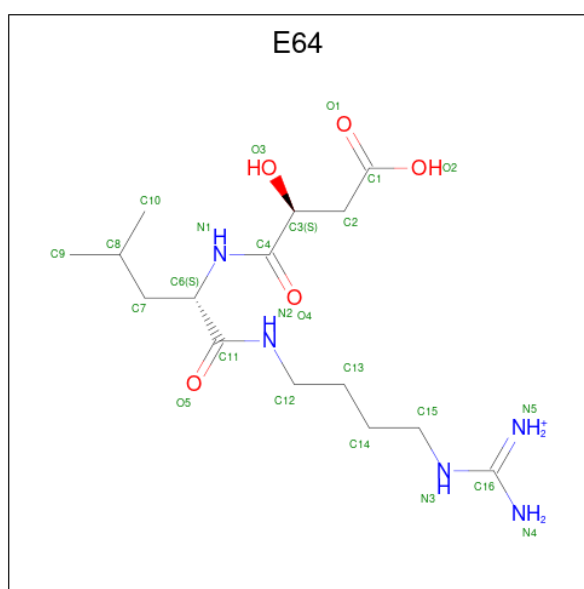
Mol	Chain	Residues	Atoms				AltConf
2	A	1	Total 25	C 15	N 5	O 5	0

- Molecule 3 is water.

Mol	Chain	Residues	Atoms		AltConf
3	A	10	Total 10	O 10	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: Papain
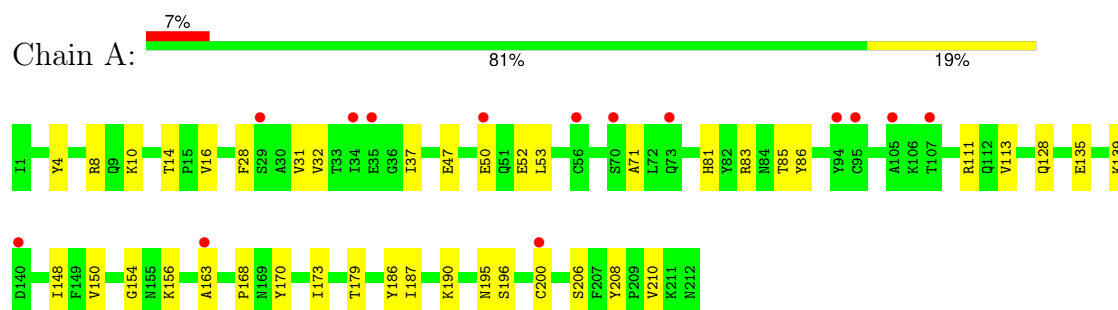
4 Data and refinement statistics ⓘ

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, α, β, γ	41.70Å 49.28Å 100.44Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	50.22 – 2.80 50.22 – 2.80	Depositor EDS
% Data completeness (in resolution range)	90.2 (50.22-2.80) 90.2 (50.22-2.80)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	1.91 (at 2.81Å)	Xtriage
Refinement program	unknown	Depositor
R, R_{free}	0.198 , 0.246 0.232 , 0.274	Depositor DCC
R_{free} test set	1026 reflections (9.97%)	wwPDB-VP
Wilson B-factor (Å^2)	29.1	Xtriage
Anisotropy	0.509	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.28 , 28.5	EDS
L-test for twinning[2]	$< |L| > = 0.44, < L^2 > = 0.27$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.86	EDS
Total number of atoms	1717	wwPDB-VP
Average B, all atoms (Å^2)	25.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 6.77% of the height of the origin peak. No significant pseudotranslation is detected.*

---

[1]Intensities estimated from amplitudes.
[2]Theoretical values of $< |L| >$, $< L^2 >$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

5 Model quality (i)

5.1 Standard geometry (i)

Bond lengths and bond angles in the following residue types are not validated in this section: E64

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	0/1728	0.66	0/2347

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	1682	0	1609	25	0
2	A	25	0	27	0	0
3	A	10	0	0	0	0
All	All	1717	0	1636	25	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 7.

All (25) 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:GLU:OE1	1:A:83:ARG:NH1	2.27	0.67

Continued on next page...

Continued from previous page...

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:53:LEU:HD13	1:A:71:ALA:HB1	1.80	0.64
1:A:111:ARG:HB2	1:A:208:TYR:CZ	2.36	0.60
1:A:8:ARG:HD3	1:A:186:TYR:CZ	2.38	0.59
1:A:150:VAL:HG23	1:A:190:LYS:HG2	1.84	0.58
1:A:173:ILE:HB	1:A:187:ILE:HG23	1.86	0.57
1:A:81:HIS:HB3	1:A:85:THR:CG2	2.36	0.56
1:A:135:GLU:HG2	1:A:156:LYS:O	2.06	0.55
1:A:52:GLU:CD	1:A:81:HIS:HD1	2.15	0.49
1:A:28:PHE:O	1:A:32:VAL:HG23	2.13	0.49
1:A:4:TYR:CE1	1:A:168:PRO:HD3	2.48	0.48
1:A:52:GLU:OE1	1:A:81:HIS:N	2.34	0.46
1:A:111:ARG:HG2	1:A:210:VAL:HG21	1.95	0.46
1:A:31:VAL:HG21	1:A:50:GLU:HG3	1.97	0.46
1:A:113:VAL:HB	1:A:206:SER:HB2	1.97	0.46
1:A:16:VAL:HB	1:A:179:THR:HG22	1.97	0.45
1:A:163:ALA:HA	1:A:173:ILE:HD13	1.99	0.45
1:A:81:HIS:HB3	1:A:85:THR:HG23	1.98	0.44
1:A:10:LYS:HB3	1:A:10:LYS:HE3	1.59	0.44
1:A:139:LYS:HB3	1:A:139:LYS:HE2	1.55	0.44
1:A:14:THR:HB	1:A:47:GLU:OE1	2.18	0.43
1:A:37:ILE:HD13	1:A:128:GLN:OE1	2.19	0.43
1:A:148:ILE:HD12	1:A:170:TYR:HB3	2.01	0.43
1:A:154:GLY:O	1:A:200:CYS:HA	2.18	0.43
1:A:111:ARG:HG2	1:A:210:VAL:CG2	2.50	0.42

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	213/212 (100%)	208 (98%)	5 (2%)	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	171/170 (101%)	168 (98%)	3 (2%)	54	83

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

Mol	Chain	Res	Type
1	A	86	TYR
1	A	195	ASN
1	A	196	SER

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	142	GLN
1	A	195	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 ⓘ

1 ligand is 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	E64	A	301	1	24,24,24	0.66	0	27,30,30	0.73	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	E64	A	301	1	-	7/29/29/29	-

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (7) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	A	301	E64	C1-C2-C3-O3
2	A	301	E64	C6-C7-C8-C10
2	A	301	E64	O2-C1-C2-C3
2	A	301	E64	N1-C6-C7-C8
2	A	301	E64	O1-C1-C2-C3
2	A	301	E64	C11-C6-C7-C8
2	A	301	E64	C1-C2-C3-C4

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 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 E64 A 301

Bond lengths

Bond angles

Torsions

Rings
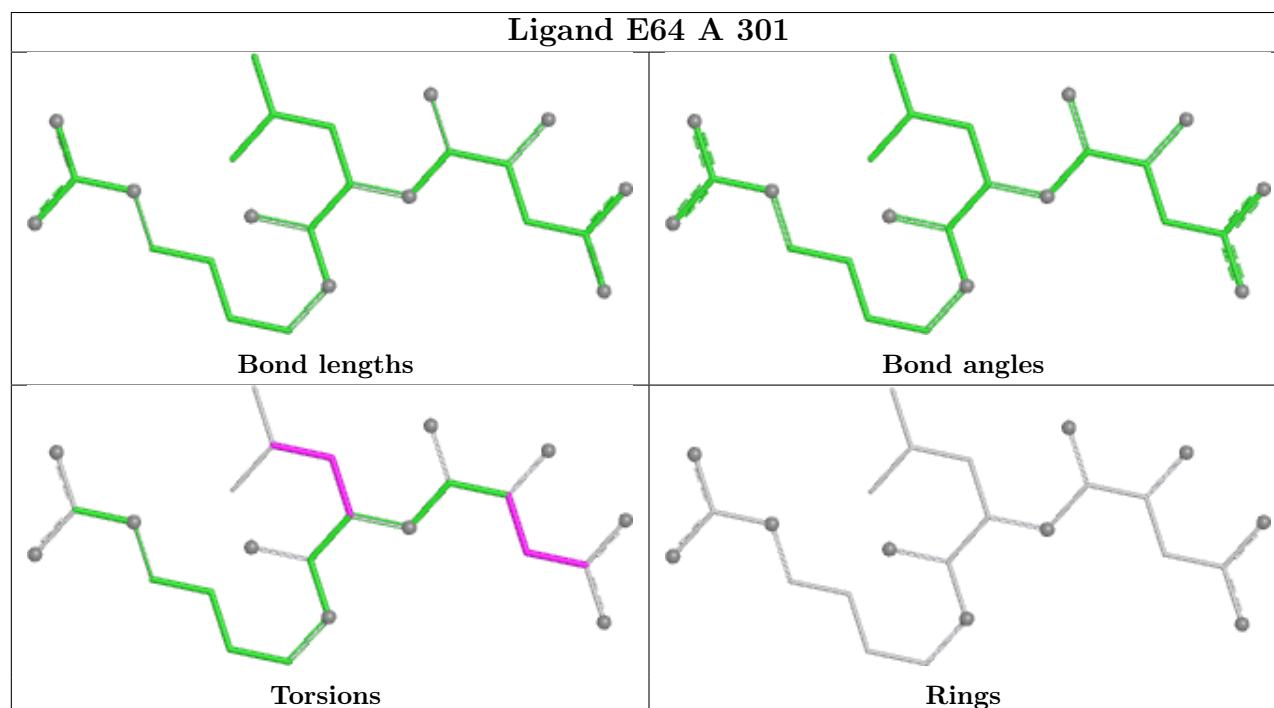
5.7 Other polymers ⓘ

There are no such residues in this entry.

5.8 Polymer linkage issues ⓘ

There are no chain breaks in this entry.