Full wwPDB EM Validation Report ⓘ

Mar 3, 2025 – 12:11 pm GMT

PDB ID	:	9F25
EMDB ID	:	EMD-50138
Title	:	Cryo-EM structure of Botulinum neurotoxin A LC-HN domain
Authors	:	Khanppnavar, B.; Leka, O.; Korkhov, V.; Kammerer, R.
Deposited on	:	2024-04-22
Resolution	:	3.70 Å(reported)
Based on initial model	:	6UL6

This is a Full wwPDB EM Validation Report for a publicly released PDB 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:

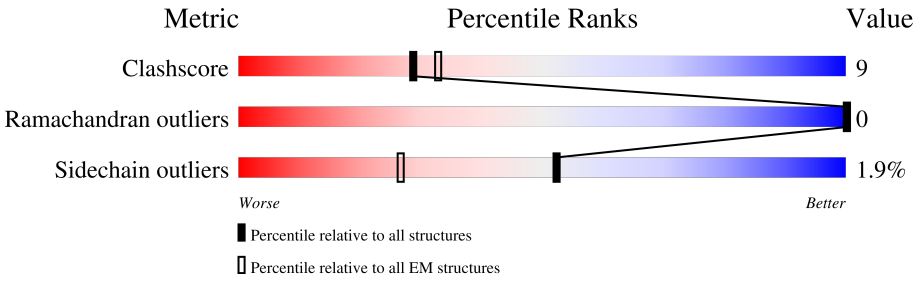
EMDB validation analysis	:	FAILED
MolProbity	:	4.02b-467
Percentile statistics	:	20231227.v01 (using entries in the PDB archive December 27th 2023)
MapQ	:	FAILED
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.41

1 Overall quality at a glance (i)

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

The reported resolution of this entry is 3.70 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)	EM structures (#Entries)
Clashscore	210492	15764
Ramachandran outliers	207382	16835
Sidechain outliers	206894	16415

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	1329	48% 14% 38%

2 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 6717 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 Botulinum neurotoxin type A.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	A	830	6717	4331	1079	1288	19	0	0

There are 39 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-16	MET	-	initiating methionine	UNP P0DPI0
A	-15	ARG	-	expression tag	UNP P0DPI0
A	-14	GLY	-	expression tag	UNP P0DPI0
A	-13	SER	-	expression tag	UNP P0DPI0
A	-12	HIS	-	expression tag	UNP P0DPI0
A	-11	HIS	-	expression tag	UNP P0DPI0
A	-10	HIS	-	expression tag	UNP P0DPI0
A	-9	HIS	-	expression tag	UNP P0DPI0
A	-8	HIS	-	expression tag	UNP P0DPI0
A	-7	HIS	-	expression tag	UNP P0DPI0
A	-6	GLY	-	expression tag	UNP P0DPI0
A	-5	SER	-	expression tag	UNP P0DPI0
A	-4	LEU	-	expression tag	UNP P0DPI0
A	-3	VAL	-	expression tag	UNP P0DPI0
A	-2	PRO	-	expression tag	UNP P0DPI0
A	-1	ARG	-	expression tag	UNP P0DPI0
A	0	GLY	-	expression tag	UNP P0DPI0
A	1	SER	-	expression tag	UNP P0DPI0
A	27	ALA	VAL	variant	UNP P0DPI0
A	224	GLN	GLU	engineered mutation	UNP P0DPI0
A	363	ALA	ARG	engineered mutation	UNP P0DPI0
A	366	PHE	TYR	engineered mutation	UNP P0DPI0
A	1158	ALA	THR	conflict	UNP P0DPI0
A	1297	VAL	-	expression tag	UNP P0DPI0
A	1298	PRO	-	expression tag	UNP P0DPI0
A	1299	PRO	-	expression tag	UNP P0DPI0
A	1300	THR	-	expression tag	UNP P0DPI0
A	1301	PRO	-	expression tag	UNP P0DPI0

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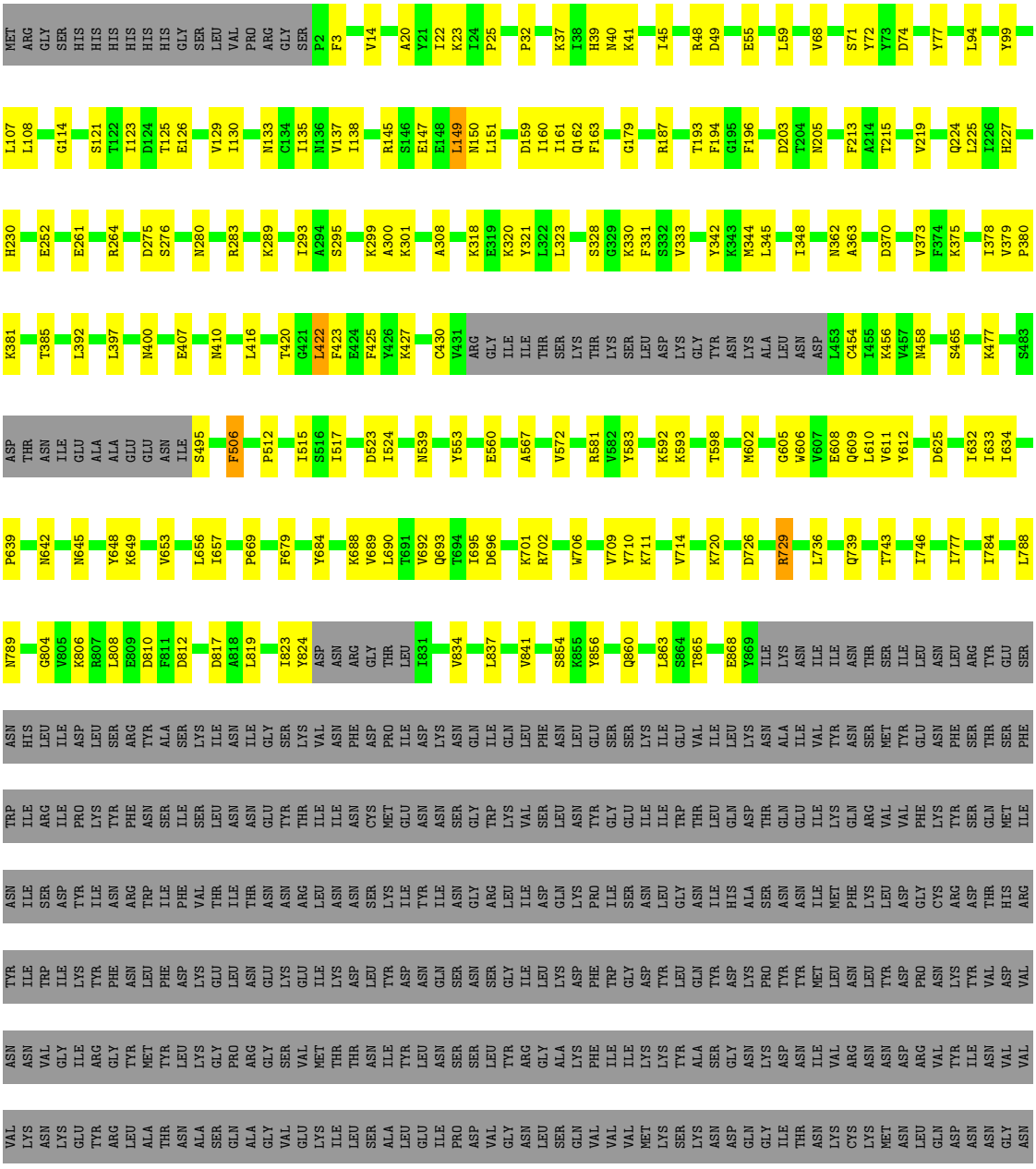
Chain	Residue	Modelled	Actual	Comment	Reference
A	1302	GLY	-	expression tag	UNP P0DPI0
A	1303	SER	-	expression tag	UNP P0DPI0
A	1304	ALA	-	expression tag	UNP P0DPI0
A	1305	TRP	-	expression tag	UNP P0DPI0
A	1306	SER	-	expression tag	UNP P0DPI0
A	1307	HIS	-	expression tag	UNP P0DPI0
A	1308	PRO	-	expression tag	UNP P0DPI0
A	1309	GLN	-	expression tag	UNP P0DPI0
A	1310	PHE	-	expression tag	UNP P0DPI0
A	1311	GLU	-	expression tag	UNP P0DPI0
A	1312	LYS	-	expression tag	UNP P0DPI0

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. 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: Botulinum neurotoxin type A

Chain A:



ASP ILE GLY PHE ILE GLY PHE HIS GLN PHE ASN ASN ILE ALA LYS LEU VAL ALA SER ASN TRP TYR ASN ARG GLN ILE GLU ARG SER SER ARG THR LEU GLY CYS SER TRP GLU PHE ILE PRO VAL ASP ASP GLY TRP GLY GLU ARG PRO LEU VAL PRO PRO THR PRO GLY SER ALA TRP

SER HIS PRO GLN PHE GLU LYS

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	121176	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	TFS KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	80	Depositor
Minimum defocus (nm)	600	Depositor
Maximum defocus (nm)	2400	Depositor
Magnification	Not provided	
Image detector	GATAN K3 BIOCONTINUUM (6k x 4k)	Depositor

5 Model quality

5.1 Standard geometry

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	0/6862	0.46	0/9294

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

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	6717	0	6618	123	0
All	All	6717	0	6618	123	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 (123) 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:430:CYS:HB3	1:A:454:CYS:HA	1.50	0.93
1:A:633:ILE:HG13	1:A:656:LEU:HD23	1.60	0.83
1:A:107:LEU:HD21	1:A:225:LEU:HB3	1.67	0.77
1:A:726:ASP:OD1	1:A:729:ARG:NH2	2.28	0.67
1:A:605:GLY:O	1:A:609:GLN:HG3	1.95	0.66

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:40:ASN:ND2	1:A:512:PRO:O	2.30	0.65
1:A:22:ILE:HG22	1:A:137:VAL:HG12	1.78	0.63
1:A:743:THR:HA	1:A:746:ILE:HD12	1.82	0.61
1:A:789:ASN:HB3	1:A:865:THR:HG21	1.82	0.61
1:A:135:ILE:HG12	1:A:149:LEU:HD13	1.82	0.60
1:A:804:GLY:O	1:A:808:LEU:HD12	2.01	0.60
1:A:230:HIS:NE2	1:A:345:LEU:O	2.24	0.60
1:A:121:SER:OG	1:A:126:GLU:OE2	2.20	0.59
1:A:48:ARG:HH11	1:A:59:LEU:HD13	1.66	0.59
1:A:48:ARG:NH2	1:A:74:ASP:O	2.35	0.59
1:A:806:LYS:NZ	1:A:810:ASP:OD2	2.36	0.58
1:A:23:LYS:HG3	1:A:138:ILE:HD11	1.85	0.57
1:A:736:LEU:HB3	1:A:777:ILE:HD12	1.86	0.57
1:A:695:ILE:HD13	1:A:837:LEU:HD22	1.88	0.56
1:A:422:LEU:O	1:A:458:ASN:ND2	2.36	0.55
1:A:608:GLU:HA	1:A:611:VAL:HG12	1.89	0.55
1:A:205:ASN:ND2	1:A:400:ASN:OD1	2.38	0.55
1:A:48:ARG:HD2	1:A:59:LEU:HD13	1.88	0.55
1:A:362:ASN:OD1	1:A:363:ALA:N	2.38	0.55
1:A:55:GLU:OE1	1:A:55:GLU:N	2.34	0.55
1:A:123:ILE:HG22	1:A:125:THR:H	1.72	0.54
1:A:710:TYR:HB3	1:A:856:TYR:CE2	2.42	0.54
1:A:425:PHE:HD1	1:A:539:ASN:HD21	1.54	0.54
1:A:130:ILE:O	1:A:133:ASN:ND2	2.41	0.53
1:A:308:ALA:HB2	1:A:515:ILE:HG12	1.91	0.53
1:A:375:LYS:HB2	1:A:416:LEU:HD11	1.91	0.53
1:A:593:LYS:NZ	1:A:609:GLN:OE1	2.33	0.53
1:A:709:VAL:HG11	1:A:808:LEU:HD11	1.90	0.53
1:A:706:TRP:CD2	1:A:808:LEU:HD22	2.44	0.52
1:A:868:GLU:OE1	1:A:868:GLU:N	2.34	0.52
1:A:583:TYR:OH	1:A:639:PRO:O	2.22	0.52
1:A:114:GLY:HA2	1:A:320:LYS:HG2	1.91	0.52
1:A:264:ARG:NH1	1:A:342:TYR:OH	2.44	0.51
1:A:817:ASP:OD1	1:A:817:ASP:N	2.43	0.51
1:A:710:TYR:HB3	1:A:856:TYR:HE2	1.74	0.50
1:A:553:TYR:CE2	1:A:642:ASN:HB2	2.46	0.50
1:A:203:ASP:OD2	1:A:860:GLN:NE2	2.44	0.50
1:A:196:PHE:O	1:A:213:PHE:N	2.34	0.50
1:A:407:GLU:O	1:A:410:ASN:ND2	2.44	0.49
1:A:688:LYS:O	1:A:692:VAL:HG23	2.13	0.49
1:A:163:PHE:O	1:A:224:GLN:NE2	2.43	0.49

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:318:LYS:HB2	1:A:331:PHE:HE2	1.77	0.49
1:A:714:VAL:HG21	1:A:856:TYR:HD2	1.78	0.49
1:A:295:SER:OG	1:A:299:LYS:NZ	2.45	0.48
1:A:642:ASN:HD21	1:A:645:ASN:HA	1.77	0.48
1:A:679:PHE:HE2	1:A:701:LYS:HB3	1.77	0.48
1:A:344:MET:HG3	1:A:348:ILE:HD12	1.95	0.48
1:A:145:ARG:NH2	1:A:147:GLU:OE2	2.34	0.48
1:A:689:VAL:HG22	1:A:693:GLN:HE21	1.79	0.48
1:A:819:LEU:O	1:A:823:ILE:HG23	2.14	0.48
1:A:193:THR:OG1	1:A:215:THR:O	2.30	0.48
1:A:94:LEU:HD21	1:A:378:ILE:HD12	1.95	0.48
1:A:130:ILE:HB	1:A:133:ASN:HD22	1.79	0.48
1:A:22:ILE:HD11	1:A:45:ILE:HD11	1.95	0.47
1:A:632:ILE:HG22	1:A:634:ILE:HG12	1.96	0.47
1:A:649:LYS:O	1:A:653:VAL:HG23	2.15	0.47
1:A:702:ARG:HD2	1:A:706:TRP:HD1	1.79	0.46
1:A:219:VAL:HG11	1:A:362:ASN:ND2	2.30	0.46
1:A:344:MET:SD	1:A:506:PHE:HE2	2.39	0.46
1:A:323:LEU:HD22	1:A:333:VAL:HG22	1.97	0.46
1:A:669:PRO:HB3	1:A:720:LYS:HB3	1.96	0.46
1:A:553:TYR:CD2	1:A:642:ASN:HB2	2.50	0.46
1:A:692:VAL:O	1:A:695:ILE:HG22	2.16	0.46
1:A:129:VAL:HG22	1:A:133:ASN:HD21	1.81	0.46
1:A:25:PRO:HA	1:A:524:ILE:HB	1.98	0.46
1:A:711:LYS:O	1:A:711:LYS:HD3	2.16	0.46
1:A:129:VAL:HG11	1:A:517:ILE:HD11	1.99	0.45
1:A:423:PHE:HD2	1:A:456:LYS:HE3	1.82	0.45
1:A:194:PHE:HD1	1:A:370:ASP:OD2	2.00	0.45
1:A:392:LEU:HA	1:A:392:LEU:HD23	1.82	0.45
1:A:854:SER:HA	1:A:863:LEU:HD11	1.99	0.45
1:A:300:ALA:O	1:A:301:LYS:HD3	2.16	0.45
1:A:606:TRP:CD1	1:A:610:LEU:HG	2.52	0.45
1:A:328:SER:O	1:A:330:LYS:NZ	2.50	0.44
1:A:702:ARG:NH2	1:A:812:ASP:OD2	2.38	0.44
1:A:784:ILE:HD13	1:A:784:ILE:HA	1.84	0.44
1:A:39:HIS:CD2	1:A:40:ASN:H	2.36	0.44
1:A:837:LEU:O	1:A:841:VAL:HG12	2.17	0.44
1:A:179:GLY:O	1:A:321:TYR:OH	2.23	0.43
1:A:381:LYS:NZ	1:A:385:THR:OG1	2.51	0.43
1:A:523:ASP:OD1	1:A:523:ASP:N	2.52	0.43
1:A:68:VAL:O	1:A:420:THR:HG21	2.18	0.43

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:49:ASP:OD2	1:A:187:ARG:NE	2.37	0.42
1:A:381:LYS:HB3	1:A:381:LYS:HE3	1.67	0.42
1:A:684:TYR:CD2	1:A:690:LEU:HG	2.54	0.42
1:A:99:TYR:HD1	1:A:108:LEU:HD12	1.85	0.42
1:A:252:GLU:O	1:A:427:LYS:NZ	2.40	0.42
1:A:108:LEU:HD23	1:A:108:LEU:HA	1.85	0.42
1:A:689:VAL:HG13	1:A:693:GLN:HE22	1.85	0.42
1:A:41:LYS:HB3	1:A:150:ASN:HD22	1.85	0.42
1:A:477:LYS:HB2	1:A:477:LYS:HE3	1.69	0.42
1:A:560:GLU:OE1	1:A:560:GLU:N	2.52	0.42
1:A:225:LEU:HD23	1:A:225:LEU:HA	1.89	0.41
1:A:379:VAL:HB	1:A:380:PRO:HD3	2.01	0.41
1:A:289:LYS:O	1:A:293:ILE:HG12	2.19	0.41
1:A:695:ILE:HG13	1:A:819:LEU:HD21	2.02	0.41
1:A:777:ILE:HD13	1:A:777:ILE:HA	1.85	0.41
1:A:280:ASN:OD1	1:A:283:ARG:NH1	2.53	0.41
1:A:14:VAL:HG13	1:A:20:ALA:HA	2.03	0.41
1:A:77:TYR:HD2	1:A:160:ILE:HD11	1.85	0.41
1:A:739:GLN:O	1:A:743:THR:HG22	2.19	0.41
1:A:159:ASP:HB3	1:A:162:GLN:HB3	2.01	0.41
1:A:32:PRO:HB3	1:A:138:ILE:HD13	2.03	0.41
1:A:72:TYR:HB2	1:A:373:VAL:HG11	2.03	0.41
1:A:252:GLU:HG2	1:A:465:SER:HB2	2.02	0.41
1:A:344:MET:HA	1:A:348:ILE:HB	2.03	0.41
1:A:553:TYR:CZ	1:A:572:VAL:HG21	2.56	0.41
1:A:567:ALA:HB3	1:A:581:ARG:HD3	2.03	0.41
1:A:598:THR:HG21	1:A:602:MET:HB2	2.03	0.41
1:A:653:VAL:O	1:A:657:ILE:HG12	2.21	0.41
1:A:151:LEU:HD23	1:A:151:LEU:HA	1.92	0.40
1:A:823:ILE:HD12	1:A:834:VAL:HG13	2.02	0.40
1:A:701:LYS:HD2	1:A:701:LYS:HA	1.79	0.40
1:A:71:SER:HA	1:A:161:ILE:HD11	2.03	0.40
1:A:275:ASP:OD1	1:A:276:SER:N	2.50	0.40
1:A:123:ILE:HB	1:A:126:GLU:HB3	2.02	0.40
1:A:788:LEU:HD23	1:A:788:LEU:HA	1.87	0.40
1:A:227:HIS:CE1	1:A:261:GLU:HG3	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 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	822/1329 (62%)	806 (98%)	16 (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 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	747/1204 (62%)	733 (98%)	14 (2%)	52 70

All (14) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	3	PHE
1	A	37	LYS
1	A	149	LEU
1	A	397	LEU
1	A	422	LEU
1	A	495	SER
1	A	506	PHE
1	A	592	LYS
1	A	612	TYR
1	A	625	ASP
1	A	648	TYR
1	A	696	ASP
1	A	729	ARG

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Mol	Chain	Res	Type
1	A	824	TYR

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (11) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	39	HIS
1	A	67	GLN
1	A	133	ASN
1	A	205	ASN
1	A	400	ASN
1	A	533	ASN
1	A	539	ASN
1	A	642	ASN
1	A	693	GLN
1	A	739	GLN
1	A	763	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

There are no ligands in this entry.

5.7 Other polymers

There are no such residues in this entry.

5.8 Polymer linkage issues (i)

There are no chain breaks in this entry.