Full wwPDB X-ray Structure Validation Report ⓘ

Feb 19, 2016 – 09:50 PM GMT

PDB ID	:	5BQN
Title	:	Crystal structure of the LHn fragment of botulinum neurotoxin type D, mutant H233Y E230Q
Authors	:	Masuyer, G.; Davies, J.R.; Moore, K.; Chaddock, J.A.; Acharya, K.R.
Deposited on	:	2015-05-29
Resolution	:	2.30 Å(reported)

This is a Full wwPDB X-ray Structure Validation Report for a publicly released PDB entry.
We welcome your comments at validation@mail.wwpdb.org
A user guide is available at
http://wwpdb.org/validation/2016/XrayValidationReportHelp
with specific help available everywhere you see the ⓘ symbol.

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

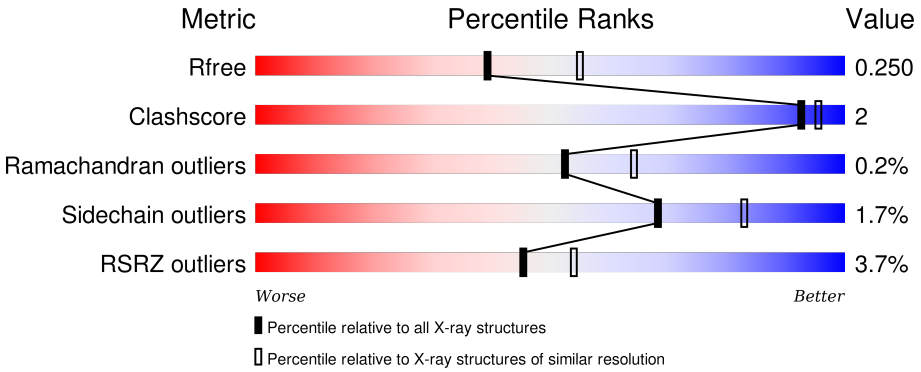
MolProbity	:	4.02b-467
Mogul	:	1.7.1 (RC1), CSD as537be (2016)
Xtriage (Phenix)	:	1.9-1692
EDS	:	rb-20026982
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
Refmac	:	5.8.0135
CCP4	:	6.5.0
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	rb-20026982

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 2.30 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}	91344	3852 (2.30-2.30)
Clashscore	102246	4452 (2.30-2.30)
Ramachandran outliers	100387	4410 (2.30-2.30)
Sidechain outliers	100360	4409 (2.30-2.30)
RSRZ outliers	91569	3857 (2.30-2.30)

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 on the lower bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria. 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% 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	885	4% 88% 6% 5%

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:

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
2	EPE	A	901	-	-	-	X

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 6920 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 Botulinum neurotoxin type D,Botulinum neurotoxin type D.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	838	Total	C	N	O	S	0	2	0
			6708	4301	1091	1298	18			

There are 37 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	230	GLN	GLU	conflict	UNP P19321
A	233	TYR	HIS	conflict	UNP P19321
A	438	VAL	-	linker	UNP P19321
A	439	ASP	-	linker	UNP P19321
A	440	LYS	-	linker	UNP P19321
A	441	SER	-	linker	UNP P19321
A	442	GLU	-	linker	UNP P19321
A	443	GLU	-	linker	UNP P19321
A	444	LYS	-	linker	UNP P19321
A	445	LEU	-	linker	UNP P19321
A	446	TYR	-	linker	UNP P19321
A	447	ASP	-	linker	UNP P19321
A	448	ASP	-	linker	UNP P19321
A	449	ASP	-	linker	UNP P19321
A	450	ASP	-	linker	UNP P19321
A	451	LYS	-	linker	UNP P19321
A	452	ASP	-	linker	UNP P19321
A	453	ARG	-	linker	UNP P19321
A	454	TRP	-	linker	UNP P19321
A	455	GLY	-	linker	UNP P19321
A	456	SER	-	linker	UNP P19321
A	457	SER	-	linker	UNP P19321
A	458	LEU	-	linker	UNP P19321
A	459	GLN	-	linker	UNP P19321
A	873	LEU	-	expression tag	UNP P19321
A	874	GLU	-	expression tag	UNP P19321
A	875	ALA	-	expression tag	UNP P19321

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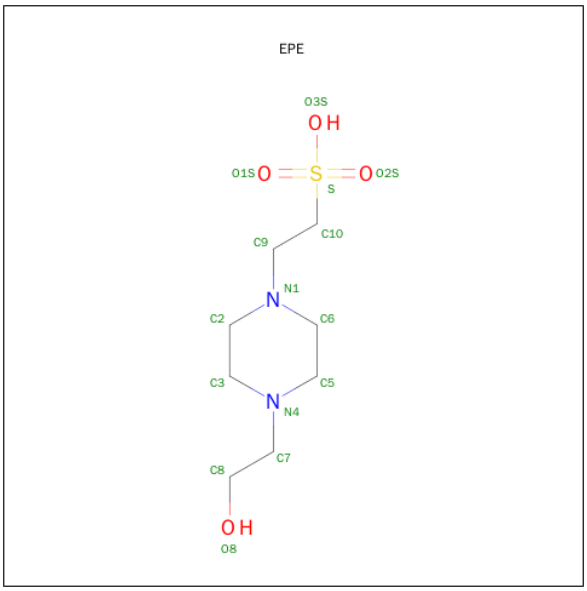
Chain	Residue	Modelled	Actual	Comment	Reference
A	876	HIS	-	expression tag	UNP P19321
A	877	HIS	-	expression tag	UNP P19321
A	878	HIS	-	expression tag	UNP P19321
A	879	HIS	-	expression tag	UNP P19321
A	880	HIS	-	expression tag	UNP P19321
A	881	HIS	-	expression tag	UNP P19321
A	882	HIS	-	expression tag	UNP P19321
A	883	HIS	-	expression tag	UNP P19321
A	884	HIS	-	expression tag	UNP P19321
A	885	HIS	-	expression tag	UNP P19321

- Molecule 2 is 4-(2-HYDROXYETHYL)-1-PIPERAZINE ETHANESULFONIC ACID (three-letter code: EPE) (formula: $C_8H_{18}N_2O_4S$).



Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
2	A	1	Total	C	N	O	S	0	0
			15	8	2	4	1		

- Molecule 3 is water.

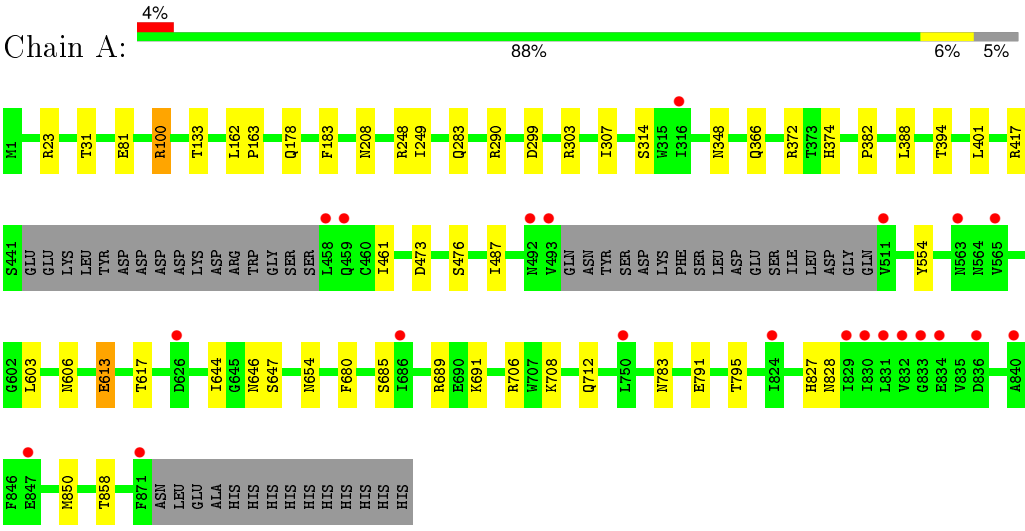
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
3	A	197	Total	O	0	0
			197	197		

3 Residue-property plots (i)

These plots are drawn for all protein, RNA and DNA chains in the entry. The first graphic for a chain summarises the proportions of errors 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: Botulinum neurotoxin type D,Botulinum neurotoxin type D

Chain A:



4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 64 2 2	Depositor
Cell constants a, b, c, α, β, γ	173.54Å 173.54Å 222.19Å 90.00° 90.00° 120.00°	Depositor
Resolution (Å)	150.00 – 2.30 89.34 – 2.30	Depositor EDS
% Data completeness (in resolution range)	98.4 (150.00-2.30) 98.4 (89.34-2.30)	Depositor EDS
R_{merge}	0.05	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	1.11 (at 2.29Å)	Xtriage
Refinement program	REFMAC 5.8.0107	Depositor
R, R_{free}	0.220 , 0.247 0.225 , 0.250	Depositor DCC
R_{free} test set	4277 reflections (5.21%)	DCC
Wilson B-factor (Å^2)	61.2	Xtriage
Anisotropy	0.256	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.31 , 50.2	EDS
Estimated twinning fraction	No twinning to report.	Xtriage
L-test for twinning[2]	$< \|L\| > = 0.51$, $< L^2 > = 0.35$	Xtriage
Outliers	2 of 86297 reflections (0.002%)	Xtriage
F_o,F_c correlation	0.96	EDS
Total number of atoms	6920	wwPDB-VP
Average B, all atoms (Å^2)	78.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.06% 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, 0.375 respectively for untwinned datasets, and 0.333, 0.2 for perfectly twinned datasets.

5 Model quality

5.1 Standard geometry

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

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	0.57	0/6858	0.73	3/9318 (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	A	0	1

There are no bond length outliers.

All (3) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	100	ARG	NE-CZ-NH2	-7.53	116.53	120.30
1	A	100	ARG	NE-CZ-NH1	6.87	123.73	120.30
1	A	417	ARG	NE-CZ-NH2	-5.43	117.58	120.30

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	691	LYS	Peptide

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	6708	0	6548	26	0
2	A	15	0	18	0	0
3	A	197	0	0	2	0
All	All	6920	0	6566	26	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 (26) 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:382:PRO:HG2	1:A:424:LEU:HD12	1.73	0.71
1:A:249:ILE:HA	1:A:476:SER:OG	1.96	0.65
1:A:374:HIS:ND1	3:A:1001:HOH:O	2.30	0.64
1:A:487:ILE:HD11	1:A:680:PHE:CD1	2.33	0.64
1:A:283:GLN:HE21	1:A:712:GLN:HE22	1.46	0.63
1:A:372[A]:ARG:HH11	1:A:372[A]:ARG:HB2	1.66	0.61
1:A:487:ILE:HD11	1:A:680:PHE:CG	2.38	0.58
1:A:290:ARG:HH11	1:A:348:ASN:HD22	1.53	0.55
1:A:461:ILE:HD12	1:A:554:TYR:CD1	2.42	0.55
1:A:461:ILE:HD12	1:A:554:TYR:CE1	2.45	0.51
1:A:473:ASP:O	1:A:476:SER:HB3	2.12	0.50
1:A:372[A]:ARG:HH11	1:A:372[A]:ARG:CB	2.26	0.49
1:A:133:THR:HG22	1:A:178:GLN:HG2	1.94	0.48
1:A:613:GLU:O	1:A:617:THR:HG22	2.13	0.48
1:A:644:ILE:O	1:A:647:SER:HB2	2.17	0.45
1:A:100:ARG:HD2	1:A:366:GLN:O	2.18	0.44
1:A:791:GLU:O	1:A:795:THR:HG23	2.19	0.43
1:A:23:ARG:NH1	1:A:31:THR:O	2.51	0.42
1:A:299:ASP:HB3	3:A:1039:HOH:O	2.18	0.42
1:A:603:LEU:HB2	1:A:606:ASN:HD22	1.85	0.41
1:A:303:ARG:O	1:A:307:ILE:HG13	2.21	0.41
1:A:394:THR:HG23	1:A:401:LEU:HD21	2.03	0.41
1:A:388:LEU:HD23	1:A:388:LEU:HA	1.96	0.40
1:A:708:LYS:HE3	1:A:858:THR:HG21	2.03	0.40
1:A:685:SER:O	1:A:827:HIS:NE2	2.40	0.40
1:A:162:LEU:HB3	1:A:163:PRO:HD2	2.03	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	834/885 (94%)	803 (96%)	29 (4%)	2 (0%)	52 64

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	439	ASP
1	A	689	ARG

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	754/823 (92%)	741 (98%)	13 (2%)	68 83

All (13) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	81	GLU
1	A	183	PHE
1	A	208	ASN
1	A	248	ARG
1	A	314	SER
1	A	613	GLU
1	A	646	ASN
1	A	654	ASN
1	A	706	ARG

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Mol	Chain	Res	Type
1	A	783	ASN
1	A	828	ASN
1	A	845	SER
1	A	850	MET

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (9) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	230	GLN
1	A	283	GLN
1	A	348	ASN
1	A	374	HIS
1	A	606	ASN
1	A	654	ASN
1	A	783	ASN
1	A	786	ASN
1	A	859	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 carbohydrates 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	EPE	A	901	-	15,15,15	1.53	1 (6%)	19,20,20	7.46	7 (36%)

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	EPE	A	901	-	-	0/9/19/19	0/1/1/1

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	901	EPE	C10-S	-5.52	1.69	1.77

All (7) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	A	901	EPE	O2S-S-C10	-23.92	89.97	106.87
2	A	901	EPE	O1S-S-C10	-17.57	94.45	106.87
2	A	901	EPE	O3S-S-C10	-11.34	81.43	104.99
2	A	901	EPE	O2S-S-O1S	2.01	119.63	113.96
2	A	901	EPE	C9-C10-S	2.13	119.61	112.61
2	A	901	EPE	O3S-S-O1S	3.90	119.89	111.26
2	A	901	EPE	O3S-S-O2S	4.08	120.30	111.26

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

No monomer is involved in short contacts.

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.

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, 95^{th} 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	838/885 (94%)	0.17	31 (3%) 45 54	49, 73, 128, 182	0

All (31) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	440	LYS	8.2
1	A	511	VAL	7.6
1	A	829	ILE	6.5
1	A	824	ILE	4.5
1	A	871	PHE	4.5
1	A	686	ILE	4.3
1	A	843	ASN	4.3
1	A	832	VAL	4.0
1	A	439	ASP	3.8
1	A	458	LEU	3.6
1	A	492	ASN	3.6
1	A	833	GLY	3.5
1	A	580	TYR	3.4
1	A	601	ALA	3.3
1	A	565	VAL	3.3
1	A	626	ASP	3.2
1	A	459	GLN	2.9
1	A	830	ILE	2.6
1	A	840	ALA	2.5
1	A	493	VAL	2.5
1	A	842	VAL	2.4
1	A	563	ASN	2.2
1	A	570	LEU	2.2
1	A	750	LEU	2.2
1	A	847	GLU	2.2
1	A	316	ILE	2.2
1	A	834	GLU	2.2

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Mol	Chain	Res	Type	RSRZ
1	A	836	ASP	2.2
1	A	831	LEU	2.2
1	A	844	GLU	2.2
1	A	841	LYS	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 carbohydrates 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. LLDF column lists the quality of electron density of the group with respect to its neighbouring residues in protein, DNA or RNA chains. The B-factors column lists the minimum, median, 95^{th} 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	LLDF	B-factors(Å^2)	Q<0.9
2	EPE	A	901	15/15	0.84	0.23	6.04	114,119,127,129	0

6.5 Other polymers (i)

There are no such residues in this entry.