Full wwPDB X-ray Structure Validation Report (i)

Sep 23, 2023 – 08:05 PM EDT

PDB ID	:	5L21
Title	:	Crystal structure of BoNT/A receptor binding domain in complex with VHH C2
Authors	:	Yao, G.; Jin, R.
Deposited on	:	2016-07-29
Resolution	:	1.68 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
https://www.wwpdb.org/validation/2017/XrayValidationReportHelp
with specific help available everywhere you see the (i) 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 (1)) were used in the production of this report:

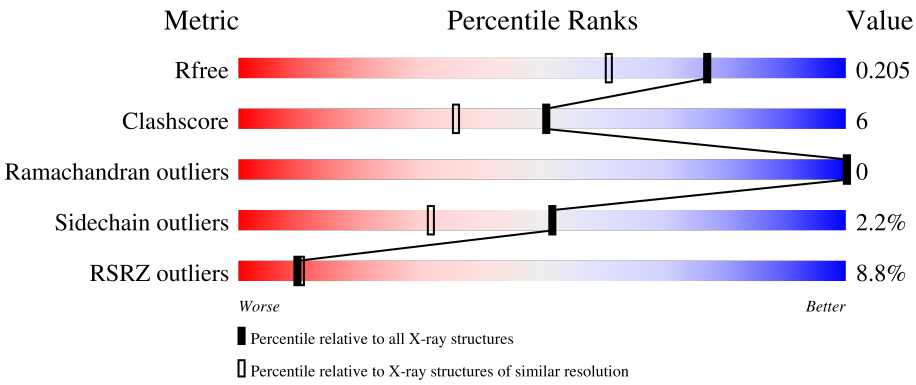
MolProbity	:	4.02b-467
Xtriage (Phenix)	:	1.13
EDS	:	2.35.1
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.35.1

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 1.68 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}	130704	6780 (1.70-1.66)
Clashscore	141614	7310 (1.70-1.66)
Ramachandran outliers	138981	7173 (1.70-1.66)
Sidechain outliers	138945	7172 (1.70-1.66)
RSRZ outliers	127900	6661 (1.70-1.66)

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 <=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	428	9% 86% 12% ••
2	B	119	8% 89% 10% •

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 5003 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 A.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	425	3499	2231	603	651	14	0	0	0

There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	869	GLY	-	cloning artifact	UNP A5HZZ9
A	870	PRO	-	cloning artifact	UNP A5HZZ9
A	871	MET	-	cloning artifact	UNP A5HZZ9
A	1158	ALA	THR	engineered mutation	UNP A5HZZ9

- Molecule 2 is a protein called VHH-C2.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
2	B	119	938	592	161	181	4	0	0	0

- Molecule 3 is water.

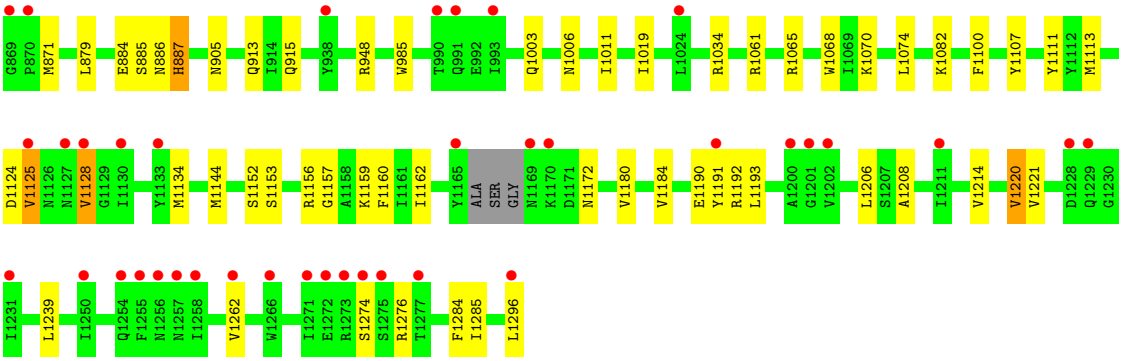
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
3	A	448	448	448	0	0
3	B	118	118	118	0	0

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.

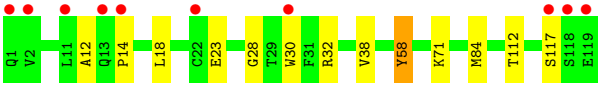
• Molecule 1: Botulinum neurotoxin type A

Chain A:



• Molecule 2: VHH-C2

Chain B:



4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α, β, γ	50.10Å 103.72Å 64.70Å 90.00° 93.77° 90.00°	Depositor
Resolution (Å)	35.99 – 1.68 35.99 – 1.68	Depositor EDS
% Data completeness (in resolution range)	99.4 (35.99-1.68) 95.4 (35.99-1.68)	Depositor EDS
R_{merge}	0.04	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	2.22 (at 1.68Å)	Xtriage
Refinement program	PHENIX 1.9_1692	Depositor
R, R_{free}	0.193 , 0.205 0.194 , 0.205	Depositor DCC
R_{free} test set	3774 reflections (5.06%)	wwPDB-VP
Wilson B-factor (Å^2)	19.6	Xtriage
Anisotropy	0.483	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.34 , 53.9	EDS
L-test for twinning[2]	$< \|L\| > = 0.49$, $< L^2 > = 0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.96	EDS
Total number of atoms	5003	wwPDB-VP
Average B, all atoms (Å^2)	36.0	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.*

[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)

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	0.30	0/3572	0.50	0/4829
2	B	0.30	0/960	0.51	0/1297
All	All	0.30	0/4532	0.50	0/6126

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	3499	0	3461	42	0
2	B	938	0	898	9	0
3	A	448	0	0	15	3
3	B	118	0	0	3	3
All	All	5003	0	4359	50	3

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 6.

All (50) 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:1134:MET:SD	1:A:1193:LEU:HD13	2.02	0.97

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:1113:MET:SD	3:A:1313:HOH:O	2.34	0.85
1:A:1134:MET:HE1	1:A:1184:VAL:HG21	1.60	0.82
1:A:1172:ASN:N	3:A:1301:HOH:O	2.04	0.78
1:A:1162:ILE:HG23	1:A:1180:VAL:HG21	1.66	0.78
1:A:1134:MET:SD	1:A:1193:LEU:CD1	2.73	0.77
1:A:1144:MET:SD	3:A:1736:HOH:O	2.43	0.76
1:A:1006:ASN:CG	3:A:1304:HOH:O	2.24	0.75
1:A:1107:TYR:O	3:A:1302:HOH:O	2.05	0.73
2:B:12:ALA:HB2	2:B:18:LEU:HD11	1.70	0.73
1:A:1006:ASN:CB	3:A:1304:HOH:O	2.36	0.73
1:A:1152:SER:OG	3:A:1304:HOH:O	2.07	0.72
1:A:1134:MET:CE	1:A:1184:VAL:HG21	2.20	0.72
1:A:1190:GLU:OE1	3:A:1305:HOH:O	2.08	0.70
1:A:1157:GLY:O	3:A:1306:HOH:O	2.12	0.67
1:A:1006:ASN:HB3	3:A:1304:HOH:O	1.97	0.65
1:A:1124:ASP:OD2	3:A:1307:HOH:O	2.16	0.63
1:A:1034:ARG:NH1	3:A:1314:HOH:O	2.31	0.62
2:B:23:GLU:HG3	2:B:84:MET:HG2	1.82	0.62
1:A:1156:ARG:NH1	3:A:1317:HOH:O	2.33	0.61
2:B:28:GLY:O	3:B:201:HOH:O	2.16	0.59
1:A:1160:PHE:HB2	3:A:1313:HOH:O	2.02	0.58
2:B:14:PRO:HD3	2:B:117:SER:O	2.05	0.57
1:A:1153:SER:OG	1:A:1156:ARG:NH2	2.38	0.56
1:A:885:SER:O	1:A:886:ASN:HB2	2.06	0.56
2:B:12:ALA:CB	2:B:18:LEU:HD11	2.37	0.54
1:A:1107:TYR:CD1	1:A:1162:ILE:HG22	2.47	0.49
1:A:1134:MET:HE3	1:A:1208:ALA:HB2	1.94	0.49
1:A:1128:VAL:HG11	1:A:1191:TYR:CE1	2.47	0.48
2:B:32:ARG:HD2	3:B:206:HOH:O	2.12	0.48
1:A:985:TRP:CD2	1:A:1019:ILE:HG21	2.49	0.48
1:A:1003:GLN:HA	1:A:1011:ILE:HD11	1.96	0.47
1:A:1192:ARG:HG2	1:A:1214:VAL:HG21	1.96	0.47
1:A:948:ARG:HB3	1:A:1068:TRP:HB2	1.97	0.46
1:A:1180:VAL:HG12	1:A:1221:VAL:O	2.16	0.46
1:A:1220:VAL:O	1:A:1239:LEU:HD12	2.15	0.45
1:A:1274:SER:OG	1:A:1276:ARG:HG2	2.17	0.45
1:A:1156:ARG:HH22	1:A:1296:LEU:HD13	1.81	0.44
1:A:884:GLU:O	1:A:887:HIS:ND1	2.49	0.43
1:A:1193:LEU:HD11	1:A:1206:LEU:HB3	1.99	0.43
1:A:905:ASN:HB3	1:A:915:GLN:HB3	2.00	0.42
2:B:38:VAL:HG22	2:B:58:TYR:HB3	2.02	0.42

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:1134:MET:CE	1:A:1193:LEU:HD13	2.50	0.41
1:A:879:LEU:HB3	1:A:1074:LEU:HB2	2.03	0.41
1:A:913:GLN:HG2	1:A:1070:LYS:HD3	2.02	0.41
1:A:1100:PHE:HB2	1:A:1285:ILE:HG12	2.03	0.41
1:A:1159:LYS:HE3	2:B:30:TRP:CZ3	2.56	0.40
2:B:71:LYS:NZ	3:B:210:HOH:O	2.49	0.40
1:A:1125:VAL:HG13	3:A:1352:HOH:O	2.22	0.40
1:A:1111:TYR:CG	1:A:1284:PHE:HB3	2.57	0.40

All (3) 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)
3:A:1748:HOH:O	3:B:316:HOH:O[2_645]	2.10	0.10
3:A:1326:HOH:O	3:B:271:HOH:O[2_645]	2.16	0.04
3:A:1741:HOH:O	3:B:250:HOH:O[1_455]	2.17	0.03

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	421/428 (98%)	407 (97%)	14 (3%)	0	100	100
2	B	117/119 (98%)	115 (98%)	2 (2%)	0	100	100
All	All	538/547 (98%)	522 (97%)	16 (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	392/393 (100%)	383 (98%)	9 (2%)	50	30
2	B	100/100 (100%)	98 (98%)	2 (2%)	55	36
All	All	492/493 (100%)	481 (98%)	11 (2%)	52	32

All (11) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	871	MET
1	A	887	HIS
1	A	1061	ARG
1	A	1065	ARG
1	A	1082	LYS
1	A	1125	VAL
1	A	1128	VAL
1	A	1220	VAL
1	A	1262	VAL
2	B	58	TYR
2	B	112	THR

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

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 monosaccharides in this entry.

5.6 Ligand geometry (i)

There are no ligands in this entry.

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	425/428 (99%)	0.49	38 (8%)	9	10	13, 31, 67, 106	0
2	B	119/119 (100%)	0.31	10 (8%)	11	11	19, 36, 63, 93	0
All	All	544/547 (99%)	0.45	48 (8%)	10	10	13, 33, 65, 106	0

All (48) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
2	B	118	SER	5.7
1	A	1255	PHE	5.3
1	A	1275	SER	5.3
1	A	1273	ARG	5.0
1	A	1256	ASN	4.8
1	A	1274	SER	4.7
1	A	1257	ASN	4.3
1	A	1229	GLN	4.2
1	A	1231	ILE	4.1
2	B	1	GLN	4.1
1	A	869	GLY	4.1
2	B	119	GLU	4.0
1	A	870	PRO	4.0
1	A	1228	ASP	4.0
1	A	1266	TRP	3.9
2	B	117	SER	3.9
1	A	1125	VAL	3.4
1	A	1165	TYR	3.4
1	A	1211	ILE	3.4
1	A	1272	GLU	3.2
2	B	2	VAL	3.1
1	A	1130	ILE	3.1
1	A	938	TYR	3.1
1	A	1170	LYS	2.9

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Mol	Chain	Res	Type	RSRZ
1	A	1202	VAL	2.9
1	A	1271	ILE	2.9
1	A	1127	ASN	2.8
1	A	1200	ALA	2.8
1	A	1128	VAL	2.7
1	A	1024	LEU	2.7
1	A	1191	TYR	2.7
1	A	1254	GLN	2.7
1	A	990	THR	2.6
1	A	1201	GLY	2.5
1	A	1250	ILE	2.4
2	B	13	GLN	2.4
1	A	1258	ILE	2.3
2	B	11	LEU	2.3
2	B	30	TRP	2.3
1	A	1133	TYR	2.3
1	A	993	ILE	2.2
2	B	22	CYS	2.2
1	A	991	GLN	2.2
1	A	1296	LEU	2.2
1	A	1169	ASN	2.1
1	A	1277	THR	2.1
2	B	14	PRO	2.1
1	A	1262	VAL	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)

There are no ligands in this entry.

6.5 Other polymers (i)

There are no such residues in this entry.