



# wwPDB X-ray Structure Validation Summary Report ⓘ

Feb 1, 2016 – 07:05 PM GMT

PDB ID : 4NP4  
Title : Clostridium difficile toxin B CROP domain in complex with FAB domains of neutralizing antibody bezlotoxumab  
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Deposited on : 2013-11-20  
Resolution : 2.89 Å(reported)

This is a wwPDB X-ray Structure Validation Summary Report for a publicly released PDB entry.  
We welcome your comments at [validation@mail.wwpdb.org](mailto: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.

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

MolProbity : 4.02b-467  
Mogul : 1.7 (RC4), CSD as536be (2015)  
Xtriage (Phenix) : 1.9-1692  
EDS : rb-20026688  
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) : trunk26865

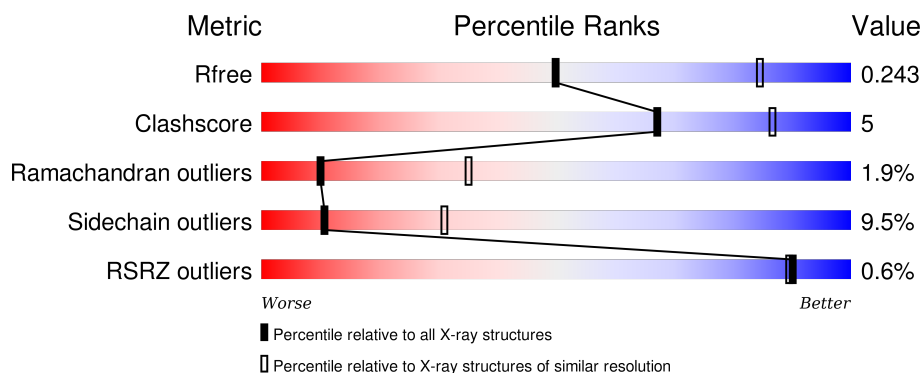
# 1 Overall quality at a glance ⓘ

The following experimental techniques were used to determine the structure:

## *X-RAY DIFFRACTION*

The reported resolution of this entry is 2.89 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	1451 (2.90-2.90)
Clashscore	102246	1668 (2.90-2.90)
Ramachandran outliers	100387	1630 (2.90-2.90)
Sidechain outliers	100360	1632 (2.90-2.90)
RSRZ outliers	91569	1456 (2.90-2.90)

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  $\geq 3$ , 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  $\leq 5\%$ . 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	275	<div> <div>78%</div> <div>17%</div> <div>..</div> </div>
2	H	222	<div> <div>76%</div> <div>18%</div> <div>..</div> </div>
2	I	222	<div> <div>3%</div> <div>76%</div> <div>20%</div> <div>.</div> </div>
3	L	215	<div> <div>84%</div> <div>12%</div> <div>..</div> </div>
3	M	215	<div> <div>87%</div> <div>11%</div> <div>..</div> </div>

## 2 Entry composition

There are 4 unique types of molecules in this entry. The entry contains 8686 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 Toxin B.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	271	Total	C	N	O	S	0	0	0
			2202	1419	334	445	4			

There are 9 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1833	MET	-	INITIATING METHIONINE	UNP P18177
A	2100	LEU	-	EXPRESSION TAG	UNP P18177
A	2101	GLU	-	EXPRESSION TAG	UNP P18177
A	2102	HIS	-	EXPRESSION TAG	UNP P18177
A	2103	HIS	-	EXPRESSION TAG	UNP P18177
A	2104	HIS	-	EXPRESSION TAG	UNP P18177
A	2105	HIS	-	EXPRESSION TAG	UNP P18177
A	2106	HIS	-	EXPRESSION TAG	UNP P18177
A	2107	HIS	-	EXPRESSION TAG	UNP P18177

- Molecule 2 is a protein called bezlotoxumab heavy chain.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	H	215	Total	C	N	O	S	0	0	0
			1628	1032	272	316	8			
2	I	221	Total	C	N	O	S	0	0	0
			1632	1030	273	321	8			

- Molecule 3 is a protein called bezlotoxumab light chain.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
3	L	213	Total	C	N	O	S	0	0	0
			1614	1007	272	331	4			
3	M	213	Total	C	N	O	S	0	0	0
			1597	995	267	331	4			

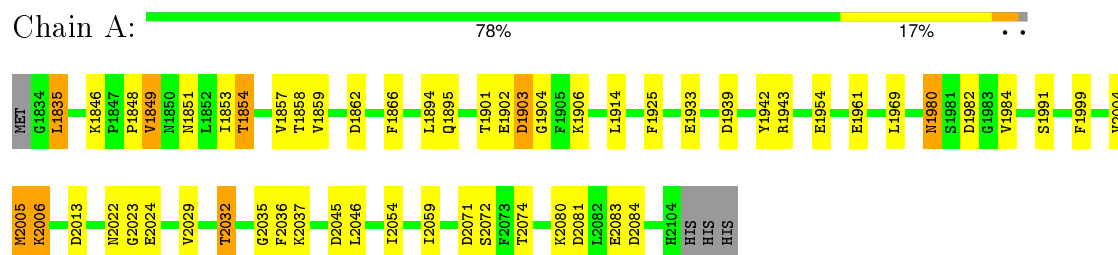
- Molecule 4 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
4	A	7	Total 7	O 7	0	0
4	I	3	Total 3	O 3	0	0
4	L	2	Total 2	O 2	0	0
4	M	1	Total 1	O 1	0	0

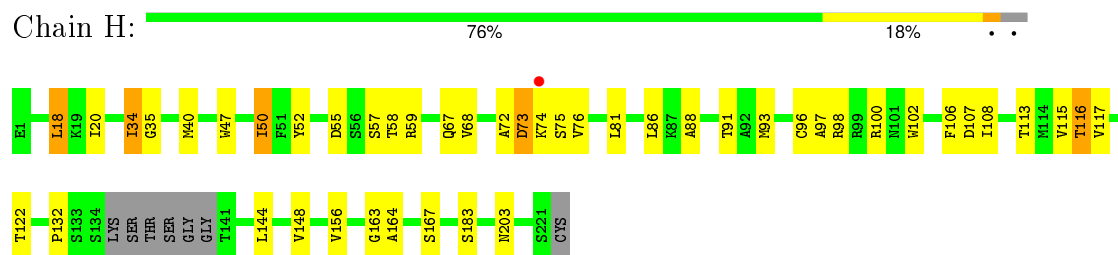
### 3 Residue-property plots

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.

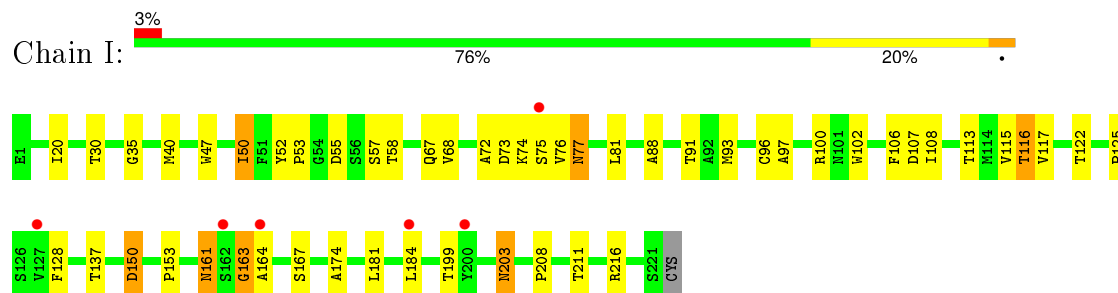
#### • Molecule 1: Toxin B



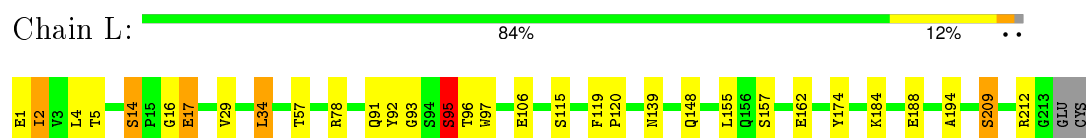
#### • Molecule 2: bezlotoxumab heavy chain



#### • Molecule 2: bezlotoxumab heavy chain



#### • Molecule 3: bezlotoxumab light chain



#### • Molecule 3: bezlotoxumab light chain

Chain M: 

87%

11%

..



## 4 Data and refinement statistics

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	79.41Å 134.66Å 102.58Å 90.00° 112.56° 90.00°	Depositor
Resolution (Å)	42.31 – 2.89 47.36 – 2.89	Depositor EDS
% Data completeness (in resolution range)	99.4 (42.31-2.89) 99.4 (47.36-2.89)	Depositor EDS
$R_{merge}$	(Not available)	Depositor
$R_{sym}$	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	2.30 (at 2.91Å)	Xtriage
Refinement program	BUSTER 2.11.4	Depositor
R, $R_{free}$	0.198 , 0.232 0.210 , 0.243	Depositor DCC
$R_{free}$ test set	931 reflections (2.14%)	DCC
Wilson B-factor (Å <sup>2</sup> )	73.6	Xtriage
Anisotropy	0.022	Xtriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.29 , 38.4	EDS
Estimated twinning fraction	0.025 for h,-k,-h-l	Xtriage
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.48$ , $\langle L^2 \rangle = 0.31$	Xtriage
Outliers	0 of 44389 reflections	Xtriage
$F_o, F_c$ correlation	0.94	EDS
Total number of atoms	8686	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	74.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.43% of the height of the origin peak. No significant pseudotranslation is detected.*

<sup>1</sup>Intensities estimated from amplitudes.

<sup>2</sup>Theoretical values of  $\langle |L| \rangle$ ,  $\langle L^2 \rangle$  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

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	0.55	0/2265	0.83	1/3065 (0.0%)
2	H	0.54	0/1671	0.86	2/2274 (0.1%)
2	I	0.53	0/1676	0.85	3/2290 (0.1%)
3	L	0.52	0/1650	0.81	1/2248 (0.0%)
3	M	0.51	0/1633	0.74	0/2229
All	All	0.53	0/8895	0.82	7/12106 (0.1%)

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
3	L	0	2

There are no bond length outliers.

The worst 5 of 7 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	I	72	ALA	C-N-CA	9.11	144.47	121.70
3	L	95	SER	C-N-CA	8.90	143.95	121.70
2	H	72	ALA	C-N-CA	5.89	136.42	121.70
2	I	163	GLY	N-CA-C	-5.79	98.62	113.10
1	A	1849	VAL	N-CA-CB	5.53	123.66	111.50

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
3	L	95	SER	Mainchain,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	2202	0	1981	23	0
2	H	1628	0	1582	17	0
2	I	1632	0	1545	22	0
3	L	1614	0	1531	13	0
3	M	1597	0	1489	7	0
4	A	7	0	0	0	0
4	I	3	0	0	0	0
4	L	2	0	0	0	0
4	M	1	0	0	0	0
All	All	8686	0	8128	80	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 5.

The worst 5 of 80 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:1901:THR:HG21	1:A:1906:LYS:HE3	1.59	0.85
2:I:52:TYR:CD2	2:I:55:ASP:HB2	2.21	0.75
2:H:91:THR:HG23	2:H:116:THR:HA	1.70	0.73
3:L:93:GLY:O	3:L:96:THR:HB	1.89	0.72
1:A:1901:THR:HG21	1:A:1906:LYS:CE	2.21	0.71

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 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	269/275 (98%)	243 (90%)	22 (8%)	4 (2%)	13	42
2	H	211/222 (95%)	192 (91%)	13 (6%)	6 (3%)	6	24
2	I	219/222 (99%)	195 (89%)	16 (7%)	8 (4%)	4	17
3	L	211/215 (98%)	199 (94%)	11 (5%)	1 (0%)	34	71
3	M	211/215 (98%)	199 (94%)	10 (5%)	2 (1%)	21	57
All	All	1121/1149 (98%)	1028 (92%)	72 (6%)	21 (2%)	10	35

5 of 21 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	1849	VAL
2	H	73	ASP
2	H	164	ALA
2	I	73	ASP
2	I	75	SER

### 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 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	229/234 (98%)	200 (87%)	29 (13%)	5	16
2	H	182/188 (97%)	165 (91%)	17 (9%)	11	32
2	I	178/188 (95%)	165 (93%)	13 (7%)	17	45
3	L	179/186 (96%)	164 (92%)	15 (8%)	14	37
3	M	175/186 (94%)	159 (91%)	16 (9%)	12	34
All	All	943/982 (96%)	853 (90%)	90 (10%)	11	31

5 of 90 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	H	98	ARG
2	I	67	GLN
3	M	108	LYS

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Mol	Chain	Res	Type
2	H	108	ILE
2	H	203	ASN

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 10 such sidechains are listed below:

Mol	Chain	Res	Type
2	I	39	GLN
2	I	65	GLN
2	I	177	GLN
1	A	2042	HIS
2	I	77	ASN

### 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 carbohydrates 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, 95<sup>th</sup> 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 <sup>2</sup> )	Q<0.9
1	A	271/275 (98%)	-0.17	0 <span>100</span> <span>100</span>	46, 66, 90, 126	0
2	H	215/222 (96%)	-0.16	1 (0%) <span>91</span> <span>90</span>	47, 69, 108, 132	0
2	I	221/222 (99%)	0.12	6 (2%) <span>58</span> <span>52</span>	54, 86, 112, 125	0
3	L	213/215 (99%)	-0.16	0 <span>100</span> <span>100</span>	44, 64, 95, 109	1 (0%)
3	M	213/215 (99%)	-0.12	0 <span>100</span> <span>100</span>	61, 80, 100, 125	1 (0%)
All	All	1133/1149 (98%)	-0.10	7 (0%) <span>90</span> <span>89</span>	44, 73, 103, 132	2 (0%)

The worst 5 of 7 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
2	I	162	SER	6.2
2	I	127	VAL	3.8
2	I	75	SER	3.5
2	I	164	ALA	2.9
2	H	74	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](#)

There are no ligands in this entry.

## 6.5 Other polymers [i](#)

There are no such residues in this entry.