Full wwPDB X-ray Structure Validation Report (i)

May 13, 2020 – 12:13 am BST

PDB ID	:	4EHK
Title	:	Allosteric Modulation of Caspase-3 through Mutagenesis
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Deposited on	:	2012-04-02
Resolution	:	1.67 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 following versions of software and data (see references (1)) were used in the production of this report:

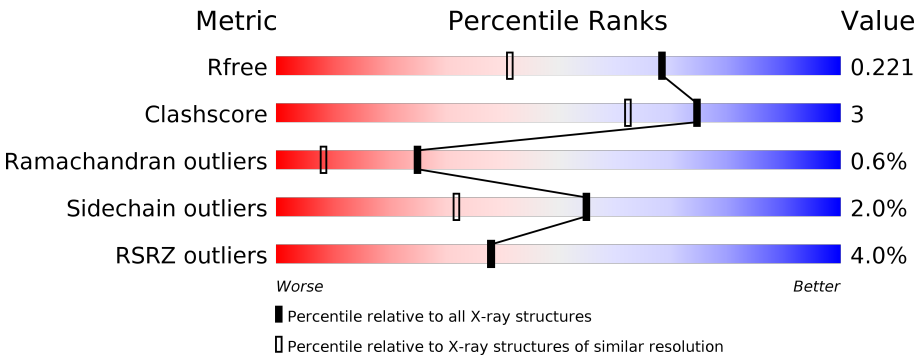
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.11
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.11

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 1.67 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	1827 (1.66-1.66)
Clashscore	141614	1931 (1.66-1.66)
Ramachandran outliers	138981	1891 (1.66-1.66)
Sidechain outliers	138945	1891 (1.66-1.66)
RSRZ outliers	127900	1791 (1.66-1.66)

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 on 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	277	3% 77% 6% • 16%
1	C	277	4% 76% 8% 16%
2	B	6	50% 33% 17%
2	D	6	33% 50% 17%

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 4218 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 Caspase-3.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	233	1864	1185	320	342	17	0	5	0
1	C	233	1879	1196	321	343	19	0	6	0

There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	124	ALA	GLU	ENGINEERED MUTATION	UNP P42574
A	197	CYS	TYR	ENGINEERED MUTATION	UNP P42574
C	124	ALA	GLU	ENGINEERED MUTATION	UNP P42574
C	197	CYS	TYR	ENGINEERED MUTATION	UNP P42574

- Molecule 2 is a protein called ACE-ASP-GLU-VAL-ASP-CHLOROMETHYLKETONE INHIBITOR.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	Cl	N	O			
2	B	6	37	21	1	4	11	0	0	1
2	D	6	37	21	1	4	11	0	0	1

- Molecule 3 is water.

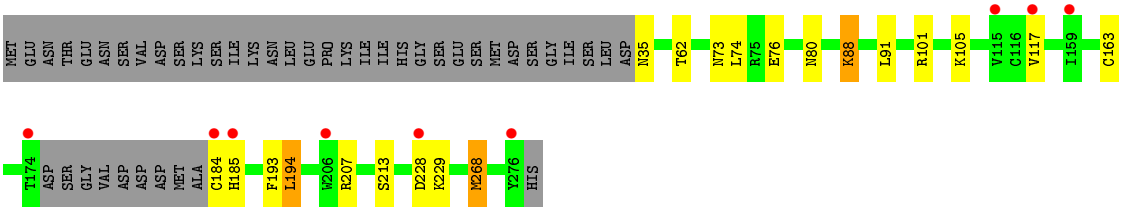
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
3	A	202	202	202	0	0
3	C	182	182	182	0	0
3	B	11	11	11	0	0
3	D	6	6	6	0	0

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 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.

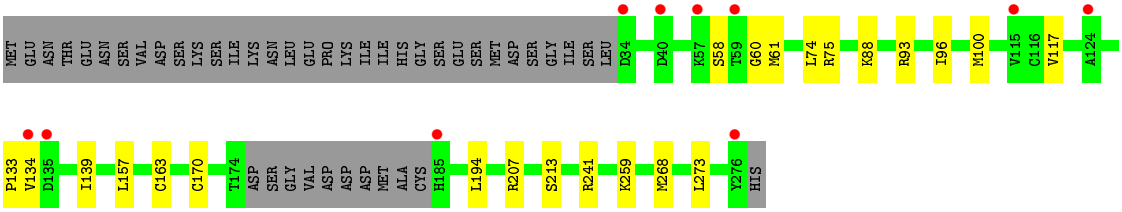
- Molecule 1: Caspase-3

Chain A:



- Molecule 1: Caspase-3

Chain C:



- Molecule 2: ACE-ASP-GLU-VAL-ASP-CHLOROMETHYLKETONE INHIBITOR

Chain B:

- Molecule 2: ACE-ASP-GLU-VAL-ASP-CHLOROMETHYLKETONE INHIBITOR

Chain D:

4 Data and refinement statistics (i)

Property	Value	Source
Space group	C 1 2 1	Depositor
Cell constants a, b, c, α, β, γ	109.82Å 96.78Å 69.18Å 90.00° 127.39° 90.00°	Depositor
Resolution (Å)	27.48 – 1.67 27.48 – 1.67	Depositor EDS
% Data completeness (in resolution range)	97.1 (27.48-1.67) 89.8 (27.48-1.67)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	2.11 (at 1.67Å)	Xtriage
Refinement program	PHENIX (phenix.refine: 1.7.3_928)	Depositor
R, R_{free}	0.196 , 0.230 0.191 , 0.221	Depositor DCC
R_{free} test set	2007 reflections (3.09%)	wwPDB-VP
Wilson B-factor (Å^2)	18.8	Xtriage
Anisotropy	0.310	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.38 , 49.8	EDS
L-test for twinning[2]	$< \|L\| > = 0.50$, $< L^2 > = 0.33$	Xtriage
Estimated twinning fraction	0.169 for -h-2*l,-k,l	Xtriage
F_o,F_c correlation	0.96	EDS
Total number of atoms	4218	wwPDB-VP
Average B, all atoms (Å^2)	24.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.73% 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: 0QE, ACE

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	0.36	0/1916	0.56	1/2575 (0.0%)
1	C	0.36	0/1931	0.55	0/2594
2	B	1.81	2/32 (6.2%)	1.57	1/43 (2.3%)
2	D	1.85	2/32 (6.2%)	1.43	1/43 (2.3%)
All	All	0.43	4/3911 (0.1%)	0.58	3/5255 (0.1%)

All (4) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	B	4	VAL	C-N	5.72	1.47	1.34
2	D	4	VAL	C-N	5.66	1.47	1.34
2	B	3	GLU	C-N	5.23	1.46	1.34
2	D	3	GLU	C-N	5.20	1.46	1.34

All (3) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	B	5	ASP	CB-CG-OD2	5.36	123.12	118.30
2	D	5	ASP	CB-CG-OD2	5.36	123.12	118.30
1	A	194	LEU	CA-CB-CG	5.01	126.83	115.30

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	1864	0	1841	12	0
1	C	1879	0	1860	12	0
2	B	37	0	26	1	0
2	D	37	0	26	2	0
3	A	202	0	0	2	0
3	B	11	0	0	0	0
3	C	182	0	0	2	0
3	D	6	0	0	0	0
All	All	4218	0	3753	24	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 3.

All (24) 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)
3:C:513:HOH:O	2:D:6:0QE:CL1	2.47	0.68
1:A:73:ASN:ND2	3:A:570:HOH:O	2.28	0.67
1:A:207:ARG:HA	1:A:213:SER:HA	1.79	0.65
1:A:184:CYS:N	3:A:534:HOH:O	2.30	0.64
1:A:76:GLU:OE1	1:A:80:ASN:ND2	2.34	0.60
1:A:163:CYS:SG	2:B:5:ASP:C	2.80	0.60
1:C:207:ARG:HA	1:C:213:SER:HA	1.83	0.60
1:A:35:ASN:OD1	1:C:241:ARG:NH2	2.37	0.58
1:C:163:CYS:SG	2:D:5:ASP:C	2.82	0.57
1:C:93:ARG:NH1	1:C:133:PRO:HD2	2.20	0.56
1:C:100[A]:MET:HG3	1:C:139:ILE:HG23	1.87	0.56
1:A:74:LEU:HD13	1:A:117:VAL:HG11	1.89	0.55
1:C:96:ILE:O	1:C:100[B]:MET:HG2	2.09	0.52
1:C:75:ARG:NH1	3:C:538:HOH:O	2.32	0.49
1:A:88:LYS:HG2	1:A:91:LEU:HD11	1.99	0.44
1:C:74:LEU:HD13	1:C:117:VAL:HG11	1.99	0.44
1:C:157:LEU:HD11	1:C:273:LEU:HD21	2.01	0.42
1:C:88:LYS:HE3	1:C:88:LYS:HB3	1.90	0.42
1:C:170[A]:CYS:SG	1:C:259:LYS:HE3	2.60	0.42
1:A:228:ASP:OD1	1:A:229:LYS:HG3	2.20	0.41
1:A:101:ARG:O	1:A:105:LYS:HG2	2.21	0.40
1:C:100[B]:MET:HG3	1:C:139:ILE:HG23	2.02	0.40
1:A:193:PHE:O	1:A:268[B]:MET:HG2	2.22	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	234/277 (84%)	229 (98%)	4 (2%)	1 (0%)	34 16
1	C	235/277 (85%)	228 (97%)	4 (2%)	3 (1%)	12 1
2	B	3/6 (50%)	3 (100%)	0	0	100 100
2	D	3/6 (50%)	3 (100%)	0	0	100 100
All	All	475/566 (84%)	463 (98%)	8 (2%)	4 (1%)	25 5

All (4) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	185	HIS
1	C	61[A]	MET
1	C	61[B]	MET
1	C	60	GLY

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	204/248 (82%)	198 (97%)	6 (3%)	42 16
1	C	206/248 (83%)	200 (97%)	6 (3%)	42 16
2	B	4/4 (100%)	4 (100%)	0	100 100

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
2	D	4/4 (100%)	4 (100%)	0	100 100
All	All	418/504 (83%)	406 (97%)	12 (3%)	55 16

All (12) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	62[A]	THR
1	A	62[B]	THR
1	A	88	LYS
1	A	194	LEU
1	A	268[A]	MET
1	A	268[B]	MET
1	C	58	SER
1	C	134	VAL
1	C	194[A]	LEU
1	C	194[B]	LEU
1	C	268[A]	MET
1	C	268[B]	MET

Some 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 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, 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	233/277 (84%)	0.02	9 (3%) 39 39	15, 22, 32, 47	4 (1%)
1	C	233/277 (84%)	0.17	10 (4%) 35 34	14, 22, 36, 50	4 (1%)
2	B	4/6 (66%)	0.04	0 100 100	18, 20, 21, 21	0
2	D	4/6 (66%)	0.07	0 100 100	20, 21, 21, 24	0
All	All	474/566 (83%)	0.09	19 (4%) 38 38	14, 22, 35, 50	8 (1%)

All (19) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	184	CYS	6.8
1	A	185	HIS	5.1
1	A	276	TYR	5.0
1	C	276	TYR	3.4
1	C	57	LYS	3.4
1	C	135	ASP	3.2
1	C	115	VAL	2.8
1	C	185	HIS	2.8
1	A	115	VAL	2.6
1	A	159	ILE	2.5
1	C	34	ASP	2.4
1	A	174	THR	2.4
1	A	117	VAL	2.4
1	C	40	ASP	2.3
1	C	134	VAL	2.2
1	C	59	THR	2.2
1	A	206	TRP	2.1
1	A	228	ASP	2.1
1	C	124	ALA	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 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.