wwPDB X-ray Structure Validation Summary Report (i)

Apr 29, 2024 – 12:55 am BST

PDB ID	:	2Y21
Title	:	The mechanisms of HAMP-mediated signaling in transmembrane receptors - the A291V mutant
Authors	:	Zeth, K.; Ferris, H.U.; Hulko, M.; Lupas, A.N.
Deposited on	:	2010-12-12
Resolution	:	2.45 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*
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 (i)) were used in the production of this report:

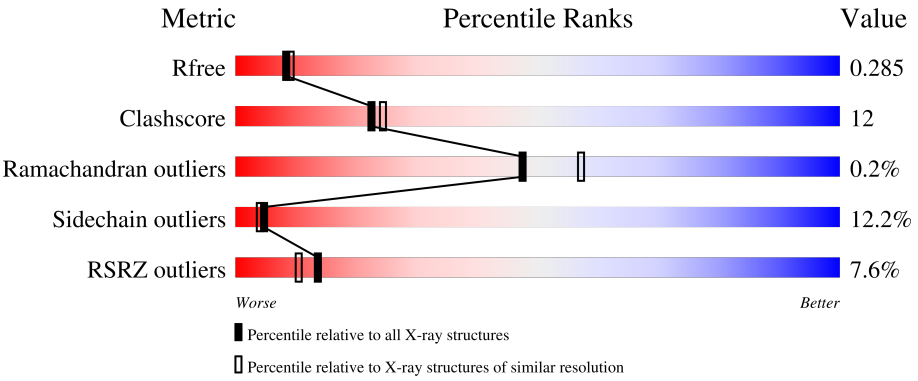
MolProbity	:	4.02b-467
Xtriage (Phenix)	:	1.13
EDS	:	2.36.2
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36.2

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 2.45 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	1544 (2.48-2.44)
Clashscore	141614	1613 (2.48-2.44)
Ramachandran outliers	138981	1598 (2.48-2.44)
Sidechain outliers	138945	1598 (2.48-2.44)
RSRZ outliers	127900	1523 (2.48-2.44)

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	56	5% 64% 21% 5% • 7%
1	B	56	7% 73% 20% • 5%
1	C	56	5% 73% 18% • 7%
1	D	56	11% 75% 20% • •
1	E	56	4% 73% 21% • •

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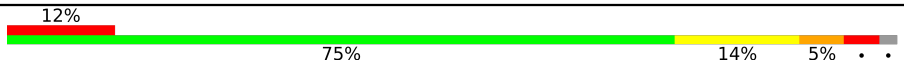
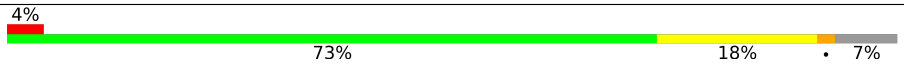
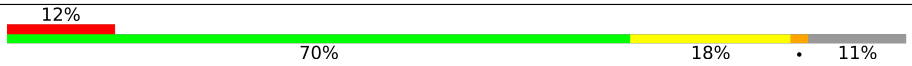
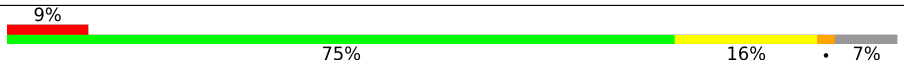
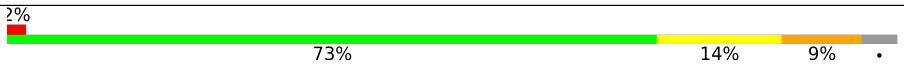
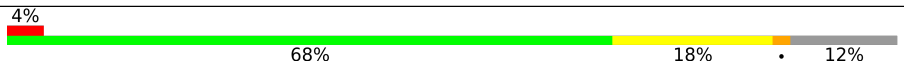
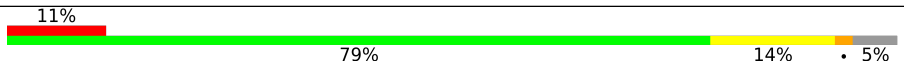
Mol	Chain	Length	Quality of chain
1	F	56	
1	G	56	
1	H	56	
1	I	56	
1	J	56	
1	K	56	
1	L	56	

2 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 4966 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 HAMP.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	52	Total	C	N	O	S	0	0	0
			404	250	76	77	1			
1	B	53	Total	C	N	O	S	0	0	0
			415	255	79	80	1			
1	C	52	Total	C	N	O	S	0	0	0
			405	249	76	79	1			
1	D	55	Total	C	N	O	S	0	0	0
			429	264	79	84	2			
1	E	54	Total	C	N	O	S	0	0	0
			418	256	78	83	1			
1	F	55	Total	C	N	O	S	0	0	0
			426	262	79	83	2			
1	G	52	Total	C	N	O		0	0	0
			401	247	76	78				
1	H	50	Total	C	N	O		0	0	0
			389	240	74	75				
1	I	52	Total	C	N	O	S	0	0	0
			406	250	76	79	1			
1	J	54	Total	C	N	O	S	0	0	0
			419	258	80	80	1			
1	K	49	Total	C	N	O		0	0	0
			381	234	73	74				
1	L	53	Total	C	N	O		0	1	0
			412	253	78	81				

There are 36 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	276	HIS	-	expression tag	UNP O28769
A	277	MET	-	expression tag	UNP O28769
A	291	VAL	ALA	engineered mutation	UNP O28769
B	276	HIS	-	expression tag	UNP O28769
B	277	MET	-	expression tag	UNP O28769

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Chain	Residue	Modelled	Actual	Comment	Reference
B	291	VAL	ALA	engineered mutation	UNP O28769
C	276	HIS	-	expression tag	UNP O28769
C	277	MET	-	expression tag	UNP O28769
C	291	VAL	ALA	engineered mutation	UNP O28769
D	276	HIS	-	expression tag	UNP O28769
D	277	MET	-	expression tag	UNP O28769
D	291	VAL	ALA	engineered mutation	UNP O28769
E	276	HIS	-	expression tag	UNP O28769
E	277	MET	-	expression tag	UNP O28769
E	291	VAL	ALA	engineered mutation	UNP O28769
F	276	HIS	-	expression tag	UNP O28769
F	277	MET	-	expression tag	UNP O28769
F	291	VAL	ALA	engineered mutation	UNP O28769
G	276	HIS	-	expression tag	UNP O28769
G	277	MET	-	expression tag	UNP O28769
G	291	VAL	ALA	engineered mutation	UNP O28769
H	276	HIS	-	expression tag	UNP O28769
H	277	MET	-	expression tag	UNP O28769
H	291	VAL	ALA	engineered mutation	UNP O28769
I	276	HIS	-	expression tag	UNP O28769
I	277	MET	-	expression tag	UNP O28769
I	291	VAL	ALA	engineered mutation	UNP O28769
J	276	HIS	-	expression tag	UNP O28769
J	277	MET	-	expression tag	UNP O28769
J	291	VAL	ALA	engineered mutation	UNP O28769
K	276	HIS	-	expression tag	UNP O28769
K	277	MET	-	expression tag	UNP O28769
K	291	VAL	ALA	engineered mutation	UNP O28769
L	276	HIS	-	expression tag	UNP O28769
L	277	MET	-	expression tag	UNP O28769
L	291	VAL	ALA	engineered mutation	UNP O28769

- Molecule 2 is water.

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
2	A	6	Total O 6 6	0	0
2	B	7	Total O 7 7	0	0
2	C	14	Total O 14 14	0	0
2	D	2	Total O 2 2	0	0

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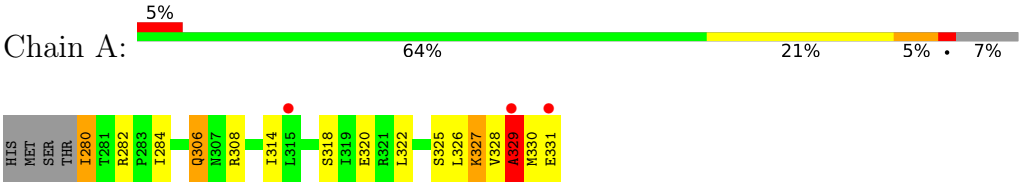
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
2	E	4	Total O 4 4	0	0
2	F	4	Total O 4 4	0	0
2	G	7	Total O 7 7	0	0
2	H	3	Total O 3 3	0	0
2	I	3	Total O 3 3	0	0
2	J	3	Total O 3 3	0	0
2	K	4	Total O 4 4	0	0
2	L	4	Total O 4 4	0	0

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.

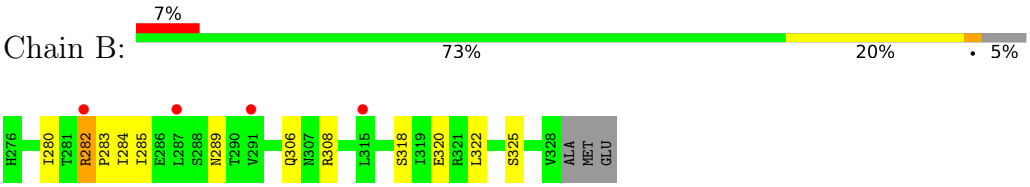
• Molecule 1: HAMP

Chain A:



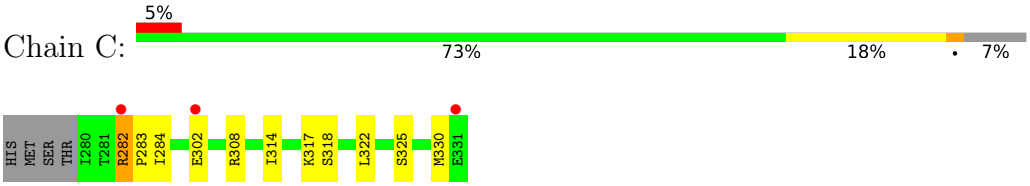
• Molecule 1: HAMP

Chain B:



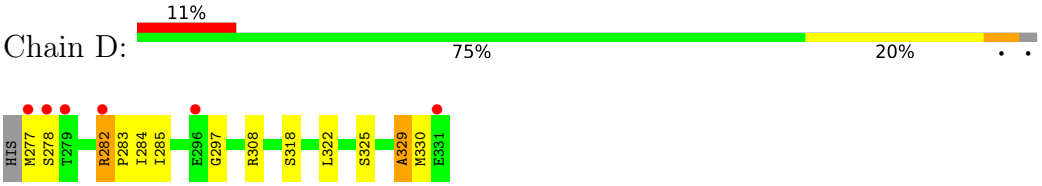
• Molecule 1: HAMP

Chain C:



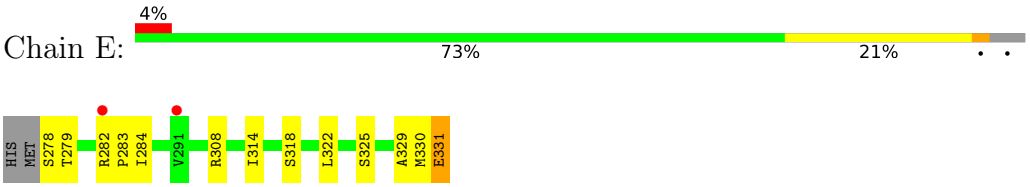
• Molecule 1: HAMP

Chain D:



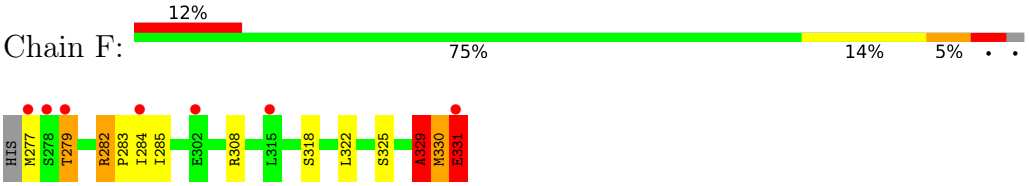
• Molecule 1: HAMP

Chain E:



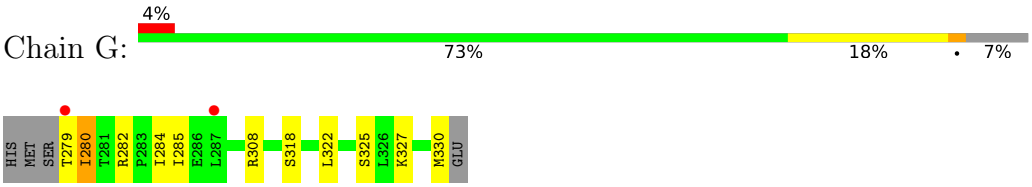
• Molecule 1: HAMP

Chain F:



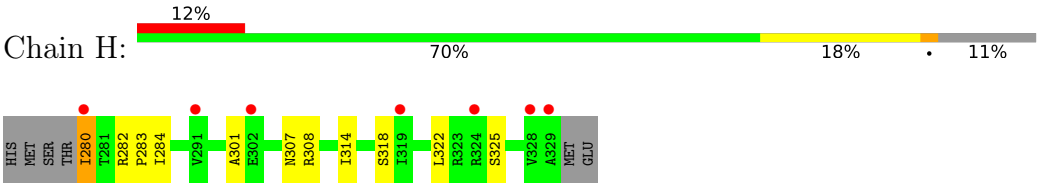
• Molecule 1: HAMP

Chain G:



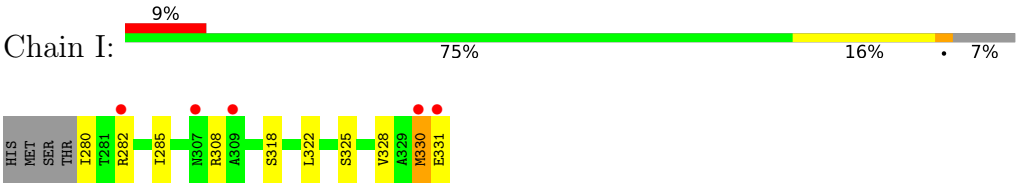
• Molecule 1: HAMP

Chain H:



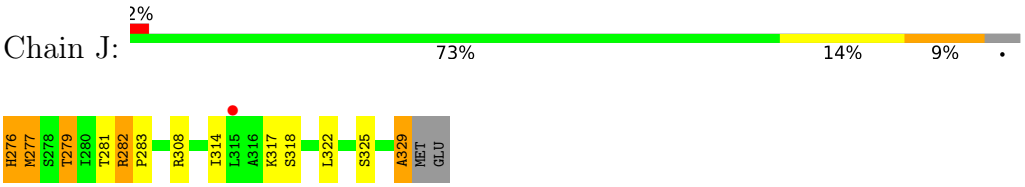
• Molecule 1: HAMP

Chain I:



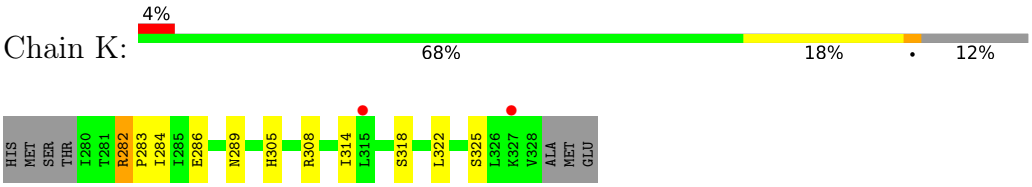
• Molecule 1: HAMP

Chain J:



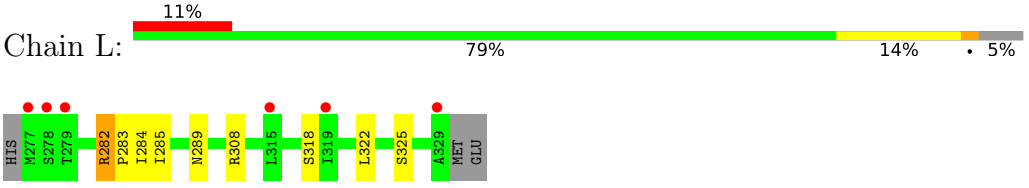
• Molecule 1: HAMP

Chain K:



• Molecule 1: HAMP

Chain L:



4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, α, β, γ	41.15Å 81.78Å 206.73Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	19.95 – 2.45 19.95 – 2.50	Depositor EDS
% Data completeness (in resolution range)	100.0 (19.95-2.45) 99.8 (19.95-2.50)	Depositor EDS
R_{merge}	0.06	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	2.33 (at 2.50Å)	Xtriage
Refinement program	REFMAC 5.5.0102	Depositor
R, R_{free}	0.246 , 0.289 0.243 , 0.285	Depositor DCC
R_{free} test set	1250 reflections (5.00%)	wwPDB-VP
Wilson B-factor (Å^2)	62.6	Xtriage
Anisotropy	0.244	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.28 , 48.0	EDS
L-test for twinning[2]	$< \|L\| > = 0.48$, $< L^2 > = 0.31$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.94	EDS
Total number of atoms	4966	wwPDB-VP
Average B, all atoms (Å^2)	72.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.30% 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

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	0.84	2/406 (0.5%)	0.68	1/546 (0.2%)
1	B	0.56	0/418	0.62	0/562
1	C	0.64	0/407	0.71	0/547
1	D	0.94	2/431 (0.5%)	1.20	5/579 (0.9%)
1	E	0.59	0/420	0.68	0/565
1	F	5.48	4/428 (0.9%)	1.82	6/575 (1.0%)
1	G	0.58	0/403	0.65	0/543
1	H	0.60	0/391	0.67	0/526
1	I	0.66	0/408	0.66	0/548
1	J	4.01	2/422 (0.5%)	1.40	1/568 (0.2%)
1	K	0.62	0/383	0.64	0/515
1	L	0.65	0/417	0.57	0/562
All	All	2.09	10/4934 (0.2%)	0.95	13/6636 (0.2%)

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	D	0	1
1	F	0	1
All	All	0	2

The worst 5 of 10 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	F	331	GLU	C-O	104.34	3.21	1.23
1	J	329	ALA	C-O	78.41	2.72	1.23
1	F	329	ALA	CA-CB	33.88	2.23	1.52
1	F	329	ALA	CA-C	23.26	2.13	1.52
1	J	329	ALA	CA-C	-21.53	0.96	1.52

The worst 5 of 13 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	F	329	ALA	CA-C-O	-34.98	46.65	120.10
1	J	329	ALA	CB-CA-C	-29.36	66.07	110.10
1	F	329	ALA	CA-C-N	-12.84	88.96	117.20
1	D	330	MET	CB-CA-C	12.59	135.59	110.40
1	F	329	ALA	N-CA-C	12.46	144.63	111.00

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	D	329	ALA	Mainchain
1	F	329	ALA	Mainchain

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	404	0	424	22	0
1	B	415	0	429	9	0
1	C	405	0	419	28	0
1	D	429	0	448	9	0
1	E	418	0	431	8	0
1	F	426	0	439	16	0
1	G	401	0	415	9	0
1	H	389	0	406	32	0
1	I	406	0	421	14	0
1	J	419	0	431	11	0
1	K	381	0	392	11	0
1	L	412	0	426	7	0
2	A	6	0	0	0	0
2	B	7	0	0	0	0
2	C	14	0	0	0	0
2	D	2	0	0	0	0
2	E	4	0	0	0	0
2	F	4	0	0	1	0
2	G	7	0	0	1	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
2	H	3	0	0	0	0
2	I	3	0	0	1	0
2	J	3	0	0	0	0
2	K	4	0	0	1	0
2	L	4	0	0	0	0
All	All	4966	0	5081	116	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 12.

The worst 5 of 116 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:C:282:ARG:HH22	1:H:307:ASN:CB	0.99	1.54
1:C:282:ARG:NH2	1:H:307:ASN:HB3	1.20	1.50
1:F:331:GLU:CA	1:F:331:GLU:C	1.78	1.47
1:C:282:ARG:NH1	1:H:307:ASN:ND2	1.67	1.36
1:C:282:ARG:HH12	1:H:307:ASN:ND2	0.90	1.35

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	50/56 (89%)	46 (92%)	3 (6%)	1 (2%)	7 5
1	B	51/56 (91%)	50 (98%)	1 (2%)	0	100 100
1	C	50/56 (89%)	49 (98%)	1 (2%)	0	100 100
1	D	53/56 (95%)	51 (96%)	2 (4%)	0	100 100
1	E	52/56 (93%)	49 (94%)	3 (6%)	0	100 100

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles
1	F	53/56 (95%)	51 (96%)	2 (4%)	0	100 100
1	G	50/56 (89%)	48 (96%)	2 (4%)	0	100 100
1	H	48/56 (86%)	47 (98%)	1 (2%)	0	100 100
1	I	50/56 (89%)	49 (98%)	1 (2%)	0	100 100
1	J	52/56 (93%)	50 (96%)	2 (4%)	0	100 100
1	K	47/56 (84%)	46 (98%)	1 (2%)	0	100 100
1	L	52/56 (93%)	51 (98%)	1 (2%)	0	100 100
All	All	608/672 (90%)	587 (96%)	20 (3%)	1 (0%)	47 57

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	329	ALA

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	44/49 (90%)	38 (86%)	6 (14%)	3 3
1	B	46/49 (94%)	42 (91%)	4 (9%)	10 11
1	C	44/49 (90%)	39 (89%)	5 (11%)	5 5
1	D	48/49 (98%)	42 (88%)	6 (12%)	4 3
1	E	46/49 (94%)	39 (85%)	7 (15%)	3 2
1	F	46/49 (94%)	39 (85%)	7 (15%)	3 2
1	G	43/49 (88%)	38 (88%)	5 (12%)	5 5
1	H	42/49 (86%)	37 (88%)	5 (12%)	5 4
1	I	44/49 (90%)	39 (89%)	5 (11%)	5 5
1	J	45/49 (92%)	38 (84%)	7 (16%)	2 1
1	K	41/49 (84%)	37 (90%)	4 (10%)	8 8

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	L	45/49 (92%)	41 (91%)	4 (9%)	9 10
All	All	534/588 (91%)	469 (88%)	65 (12%)	5 4

5 of 65 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	K	282	ARG
1	K	318	SER
1	E	318	SER
1	E	308	ARG
1	K	325	SER

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (3) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	306	GLN
1	B	306	GLN
1	H	307	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 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)

The following chains have linkage breaks:

Mol	Chain	Number of breaks
1	D	1

All chain breaks are listed below:

Model	Chain	Residue-1	Atom-1	Residue-2	Atom-2	Distance (Å)
1	D	329:ALA	C	330:MET	N	1.19

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	52/56 (92%)	0.30	3 (5%) 23 20	46, 70, 101, 112	0
1	B	53/56 (94%)	0.36	4 (7%) 14 11	46, 66, 95, 99	0
1	C	52/56 (92%)	0.15	3 (5%) 23 20	43, 68, 99, 112	1 (1%)
1	D	55/56 (98%)	0.35	6 (10%) 5 3	41, 61, 97, 106	0
1	E	54/56 (96%)	0.27	2 (3%) 41 38	42, 69, 101, 107	0
1	F	55/56 (98%)	0.68	7 (12%) 3 2	43, 66, 104, 123	1 (1%)
1	G	52/56 (92%)	0.45	2 (3%) 40 37	48, 76, 111, 121	2 (3%)
1	H	50/56 (89%)	0.52	7 (14%) 2 1	48, 68, 99, 102	0
1	I	52/56 (92%)	0.48	5 (9%) 8 5	50, 73, 105, 121	0
1	J	54/56 (96%)	0.31	1 (1%) 66 64	45, 69, 108, 123	1 (1%)
1	K	49/56 (87%)	0.41	2 (4%) 37 34	47, 75, 115, 125	0
1	L	53/56 (94%)	0.61	6 (11%) 5 3	46, 70, 111, 121	0
All	All	631/672 (93%)	0.41	48 (7%) 13 10	41, 69, 106, 125	5 (0%)

The worst 5 of 48 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	L	279	THR	5.7
1	H	280	ILE	5.6
1	L	278	SER	5.5
1	F	277	MET	4.9
1	I	330	MET	4.5

6.2 Non-standard residues in protein, DNA, RNA chains

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.