wwPDB X-ray Structure Validation Summary Report (i)

Feb 1, 2016 – 02:00 AM GMT

PDB ID	:	2F54
Title	:	Directed evolution of human T cell receptor CDR2 residues by phage display dramatically enhances affinity for cognate peptide-MHC without increasing apparent cross-reactivity
Authors	:	Rizkallah, P.J.; Jakobsen, B.K.; Dunn, S.M.; Sami, M.
Deposited on	:	2005-11-25
Resolution	:	2.70 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
http://wwpdb.org/validation/2016/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:

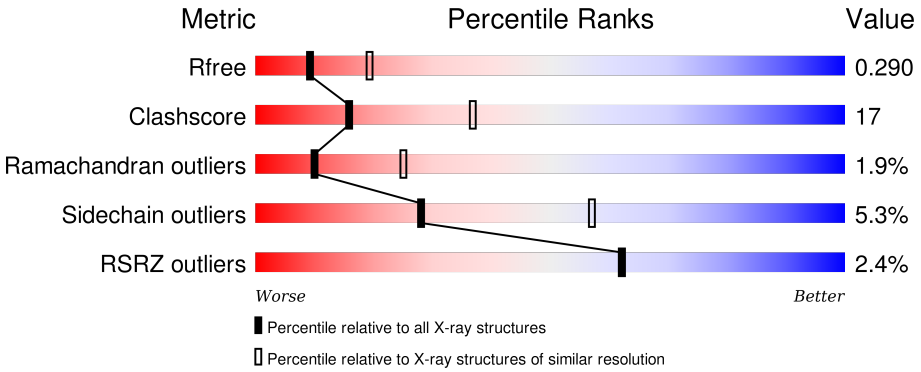
MolProbity	:	4.02b-467
Mogul	:	1.7 (RC4), CSD as536be (2015)
Xtriage (Phenix)	:	1.9-1692
EDS	:	rb-20026688
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
Refmac	:	5.8.0135
CCP4	:	6.5.0
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	trunk26865

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
X-RAY DIFFRACTION

The reported resolution of this entry is 2.70 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	2103 (2.70-2.70)
Clashscore	102246	2422 (2.70-2.70)
Ramachandran outliers	100387	2382 (2.70-2.70)
Sidechain outliers	100360	2382 (2.70-2.70)
RSRZ outliers	91569	2107 (2.70-2.70)

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	274	66% 28% 6%
1	F	274	69% 26%
2	B	100	70% 26%
2	G	100	67% 30%
3	C	9	67% 22% 11%

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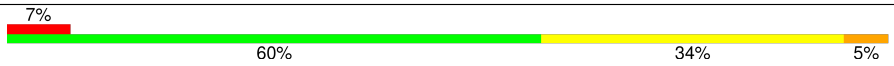
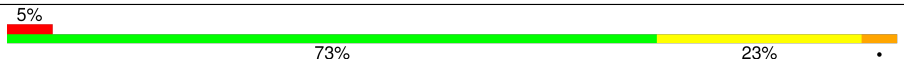
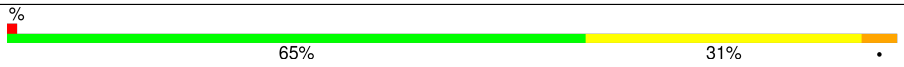
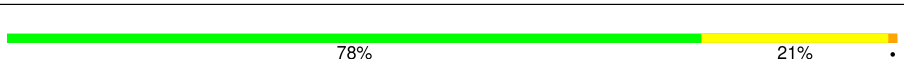
Mol	Chain	Length	Quality of chain
3	H	9	
4	D	206	
4	K	206	
5	E	241	
5	L	241	

2 Entry composition

There are 6 unique types of molecules in this entry. The entry contains 13259 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 HLA class I histocompatibility antigen.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	274	Total	C	N	O	S	0	0	0
			2238	1398	408	423	9			
1	F	274	Total	C	N	O	S	0	0	0
			2238	1398	408	423	9			

- Molecule 2 is a protein called Beta-2-microglobulin.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	B	100	Total	C	N	O	S	0	0	0
			828	524	140	158	6			
2	G	100	Total	C	N	O	S	0	0	0
			828	524	140	158	6			

There are 6 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	0	MET	-	CLONING ARTIFACT	UNP P61769
B	67	CYS	TYR	ENGINEERED	UNP P61769
B	91	CYS	LYS	ENGINEERED	UNP P61769
G	0	MET	-	CLONING ARTIFACT	UNP P61769
G	67	CYS	TYR	ENGINEERED	UNP P61769
G	91	CYS	LYS	ENGINEERED	UNP P61769

- Molecule 3 is a protein called Cancer/testis antigen 1B.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
3	C	9	Total	C	N	O	S	0	0	0
			75	49	11	13	2			
3	H	9	Total	C	N	O	S	0	0	0
			75	49	11	13	2			

- Molecule 4 is a protein called T-cell receptor alpha chain.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
4	D	205	Total	C	N	O	S	0	0	0
			1567	975	262	323	7			
4	K	206	Total	C	N	O	S	0	0	0
			1576	981	264	324	7			

- Molecule 5 is a protein called T-cell receptor beta chain.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
5	E	241	Total	C	N	O	S	0	0	0
			1902	1196	327	370	9			
5	L	241	Total	C	N	O	S	0	0	0
			1902	1196	327	370	9			

- Molecule 6 is water.

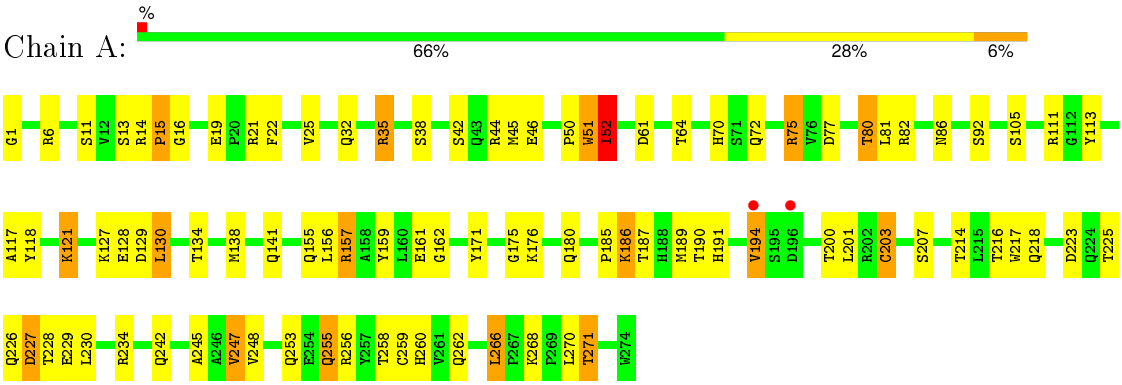
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
6	A	6	Total	O	0	0
			6	6		
6	B	1	Total	O	0	0
			1	1		
6	C	1	Total	O	0	0
			1	1		
6	D	2	Total	O	0	0
			2	2		
6	E	1	Total	O	0	0
			1	1		
6	F	8	Total	O	0	0
			8	8		
6	G	1	Total	O	0	0
			1	1		
6	K	6	Total	O	0	0
			6	6		
6	L	4	Total	O	0	0
			4	4		

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.

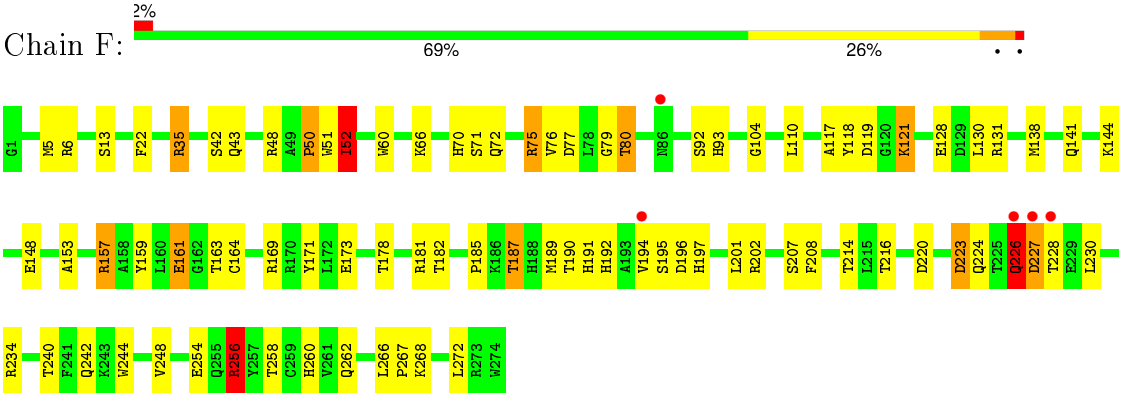
- Molecule 1: HLA class I histocompatibility antigen

Chain A:



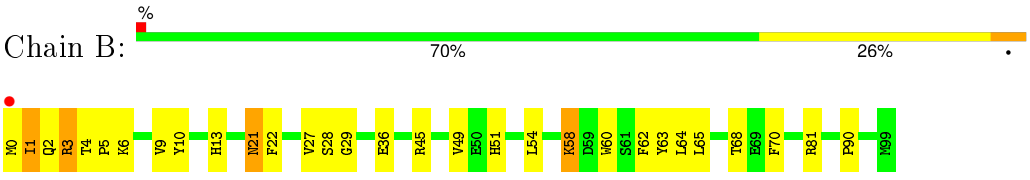
- Molecule 1: HLA class I histocompatibility antigen

Chain F:



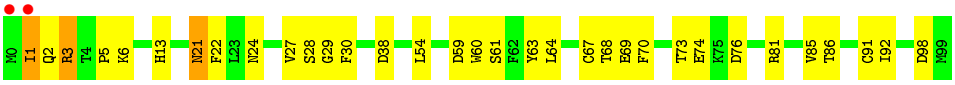
- Molecule 2: Beta-2-microglobulin

Chain B:



- Molecule 2: Beta-2-microglobulin

Chain G:

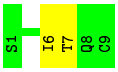


• Molecule 3: Cancer/testis antigen 1B

Chain C:

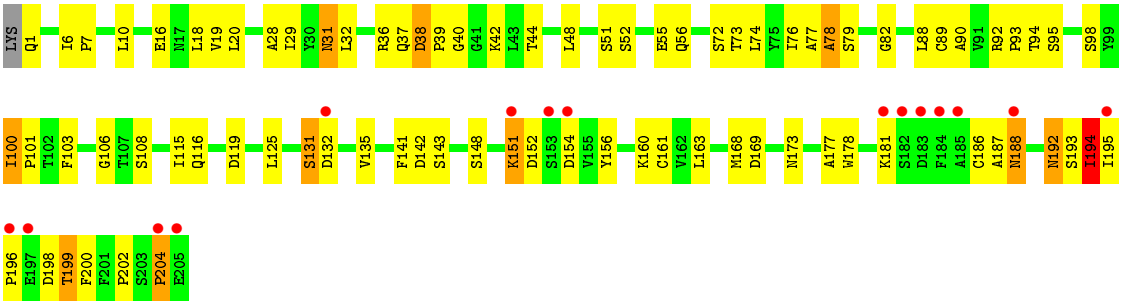
• Molecule 3: Cancer/testis antigen 1B

Chain H:



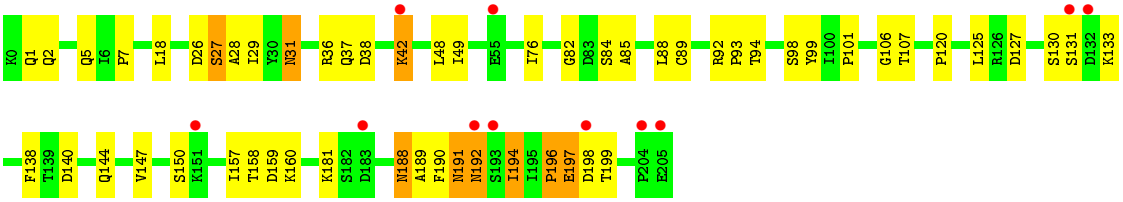
• Molecule 4: T-cell receptor alpha chain

Chain D:



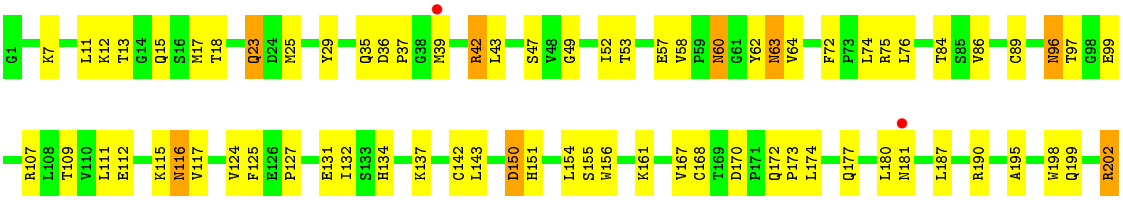
• Molecule 4: T-cell receptor alpha chain

Chain K:



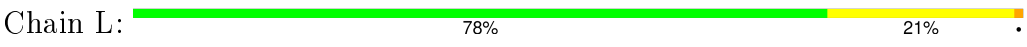
• Molecule 5: T-cell receptor beta chain

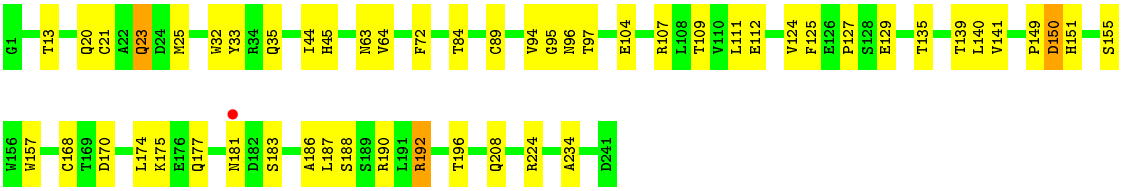
Chain E:



• Molecule 5: T-cell receptor beta chain

Chain L:





4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α, β, γ	120.02Å 53.59Å 152.83Å 90.00° 96.04° 90.00°	Depositor
Resolution (Å)	50.66 – 2.70 50.66 – 2.70	Depositor EDS
% Data completeness (in resolution range)	99.9 (50.66-2.70) 100.0 (50.66-2.70)	Depositor EDS
R_{merge}	0.13	Depositor
R_{sym}	0.13	Depositor
$< I/\sigma(I) >$ [1]	2.03 (at 2.69Å)	Xtriage
Refinement program	REFMAC 5.2.0005	Depositor
R, R_{free}	0.221 , 0.286 0.232 , 0.290	Depositor DCC
R_{free} test set	2740 reflections (5.35%)	DCC
Wilson B-factor (Å^2)	42.0	Xtriage
Anisotropy	0.563	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.31 , 43.5	EDS
Estimated twinning fraction	No twinning to report.	Xtriage
L-test for twinning[2]	$< \|L\| > = 0.48$, $< L^2 > = 0.31$	Xtriage
Outliers	1 of 53940 reflections (0.002%)	Xtriage
F_o,F_c correlation	0.91	EDS
Total number of atoms	13259	wwPDB-VP
Average B, all atoms (Å^2)	17.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The analyses of the Patterson function reveals a significant off-origin peak that is 39.84 % of the origin peak, indicating pseudo translational symmetry.The chance of finding a peak of this or larger height randomly in a structure without pseudo translational symmetry is equal to 2.9909e-04.The detected translational NCS is most likely also responsible for the elevated intensity ratio.*

[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

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	0.91	1/2303 (0.0%)	0.66	0/3125
1	F	0.85	0/2303	0.67	1/3125 (0.0%)
2	B	0.89	0/850	0.71	0/1149
2	G	0.89	2/850 (0.2%)	0.70	0/1149
3	C	0.74	0/76	0.67	0/101
3	H	0.76	0/76	0.69	0/101
4	D	0.75	1/1600 (0.1%)	0.64	1/2176 (0.0%)
4	K	0.77	0/1609	0.65	0/2187
5	E	0.79	1/1953 (0.1%)	0.67	0/2659
5	L	0.83	2/1953 (0.1%)	0.61	0/2659
All	All	0.83	7/13573 (0.1%)	0.66	2/18431 (0.0%)

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

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
5	E	89	CYS	CB-SG	-7.76	1.69	1.82
1	A	203	CYS	CB-SG	-7.76	1.69	1.82
5	L	89	CYS	CB-SG	-6.88	1.70	1.82
2	G	91	CYS	CB-SG	-6.08	1.72	1.82
2	G	1	ILE	CA-CB	5.72	1.68	1.54

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
4	D	161	CYS	CA-CB-SG	5.20	123.36	114.00
1	F	256	ARG	NE-CZ-NH1	5.18	122.89	120.30

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	2238	0	2090	72	1
1	F	2238	0	2090	77	1
2	B	828	0	791	23	0
2	G	828	0	791	29	0
3	C	75	0	79	5	0
3	H	75	0	79	3	0
4	D	1567	0	1499	72	0
4	K	1576	0	1512	57	0
5	E	1902	0	1799	84	0
5	L	1902	0	1799	45	0
6	A	6	0	0	2	0
6	B	1	0	0	0	0
6	C	1	0	0	0	0
6	D	2	0	0	1	0
6	E	1	0	0	0	0
6	F	8	0	0	4	0
6	G	1	0	0	0	0
6	K	6	0	0	0	0
6	L	4	0	0	0	0
All	All	13259	0	12529	439	1

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 17.

The worst 5 of 439 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:F:80:THR:HG21	6:F:277:HOH:O	1.51	1.09
4:D:195:ILE:O	4:D:195:ILE:HD12	1.63	0.97
4:D:186:CYS:O	4:D:188:ASN:N	1.99	0.94
4:K:196:PRO:O	4:K:197:GLU:HG3	1.68	0.94
5:E:96:ASN:HD22	5:E:97:THR:N	1.67	0.91

All (1) 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)
1:A:129:ASP:OD2	1:F:226:GLN:NE2[2_546]	2.08	0.12

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	272/274 (99%)	247 (91%)	19 (7%)	6 (2%)	8 22
1	F	272/274 (99%)	246 (90%)	21 (8%)	5 (2%)	11 27
2	B	98/100 (98%)	96 (98%)	1 (1%)	1 (1%)	19 45
2	G	98/100 (98%)	91 (93%)	7 (7%)	0	100 100
3	C	7/9 (78%)	7 (100%)	0	0	100 100
3	H	7/9 (78%)	6 (86%)	1 (14%)	0	100 100
4	D	203/206 (98%)	173 (85%)	21 (10%)	9 (4%)	3 6
4	K	204/206 (99%)	185 (91%)	14 (7%)	5 (2%)	7 18
5	E	239/241 (99%)	224 (94%)	11 (5%)	4 (2%)	11 29
5	L	239/241 (99%)	223 (93%)	15 (6%)	1 (0%)	39 69
All	All	1639/1660 (99%)	1498 (91%)	110 (7%)	31 (2%)	10 25

5 of 31 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	15	PRO
4	D	187	ALA
4	D	194	ILE
4	D	199	THR
5	E	116	ASN

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	230/230 (100%)	212 (92%)	18 (8%)	16 35
1	F	230/230 (100%)	215 (94%)	15 (6%)	21 46
2	B	95/95 (100%)	87 (92%)	8 (8%)	14 30
2	G	95/95 (100%)	89 (94%)	6 (6%)	22 48
3	C	9/9 (100%)	8 (89%)	1 (11%)	8 17
3	H	9/9 (100%)	9 (100%)	0	100 100
4	D	180/181 (99%)	174 (97%)	6 (3%)	45 76
4	K	181/181 (100%)	174 (96%)	7 (4%)	39 70
5	E	208/208 (100%)	199 (96%)	9 (4%)	35 66
5	L	208/208 (100%)	202 (97%)	6 (3%)	50 80
All	All	1445/1446 (100%)	1369 (95%)	76 (5%)	28 57

5 of 76 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
5	E	42	ARG
1	F	35	ARG
5	L	23	GLN
5	E	60	ASN
5	E	168	CYS

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 80 such sidechains are listed below:

Mol	Chain	Res	Type
5	E	116	ASN
1	F	70	HIS
5	L	60	ASN
5	E	136	GLN
5	E	199	GLN

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

There are no ligands in this entry.

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	274/274 (100%)	0.06	2 (0%) 89 90	4, 14, 28, 36	0
1	F	274/274 (100%)	0.14	5 (1%) 71 72	4, 14, 27, 37	0
2	B	100/100 (100%)	-0.12	1 (1%) 84 85	6, 13, 25, 42	0
2	G	100/100 (100%)	-0.06	2 (2%) 68 69	6, 13, 23, 42	0
3	C	9/9 (100%)	0.53	0 100 100	2, 4, 6, 7	0
3	H	9/9 (100%)	0.50	0 100 100	2, 3, 6, 7	0
4	D	205/206 (99%)	0.47	15 (7%) 18 16	6, 20, 36, 45	0
4	K	206/206 (100%)	0.26	11 (5%) 30 28	7, 20, 37, 46	0
5	E	241/241 (100%)	0.09	3 (1%) 81 81	8, 17, 30, 48	0
5	L	241/241 (100%)	0.06	1 (0%) 93 94	9, 17, 29, 47	0
All	All	1659/1660 (99%)	0.14	40 (2%) 62 62	2, 16, 33, 48	0

The worst 5 of 40 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
4	K	193	SER	7.1
4	D	205	GLU	5.7
4	K	205	GLU	4.8
1	A	196	ASP	4.7
1	F	227	ASP	4.6

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 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.