

Full wwPDB NMR Structure Validation Report (i)

May 31, 2020 – 10:11 pm BST

:	6FEH
:	Solution Structure of CaM/Kv7.2-hAB Complex
:	Bernardo-Seisdedos, G.; Villarroel, A.; Millet, O.
:	2018-01-02
	:

This is a Full wwPDB NMR 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/NMRValidationReportHelp 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:

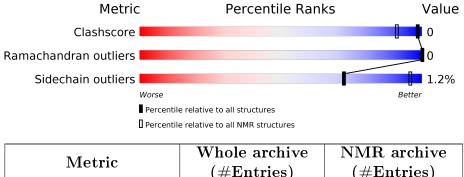
Cyrange	:	Kirchner and Güntert (2011)
$\operatorname{NmrClust}$:	Kelley et al. (1996)
$\operatorname{MolProbity}$:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
${ m ShiftChecker}$:	2.11
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: $SOLUTION\ NMR$

The overall completeness of chemical shifts assignment is 82%.

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	(# Entries)	(#Entries)
Clashscore	158937	12864
Ramachandran outliers	154571	11451
Sidechain outliers	154315	11428

The table below summarises the geometric issues observed across the polymeric chains and their fit to the experimental data. The red, orange, yellow and green segments indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria. A cyan segment indicates the fraction of residues that are not part of the well-defined cores, and 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%

Mol	Chain	Length	Quality of chain			
1	А	115	43%	·	57%	
2	В	149		93%		7% •



2 Ensemble composition and analysis (i)

This entry contains 10 models. Model 1 is the overall representative, medoid model (most similar to other models).

The following residues are included in the computation of the global validation metrics.

Well-defined (core) protein residues						
Well-defined core Residue range (total) Backbone RMSD (Å) Medoid model						
1	A:329-A:351, A:506-A:532,	0.65	1			
	B:5-B:74, B:81-B:148 (188)					

Ill-defined regions of proteins are excluded from the global statistics.

Ligands and non-protein polymers are included in the analysis.

The models can be grouped into 2 clusters and 4 single-model clusters were found.

Cluster number	Models
1	1, 2, 3
2	4, 5, 6
Single-model clusters	7; 8; 9; 10



3 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 4164 atoms, of which 2035 are hydrogens and 0 are deuteriums.

• Molecule 1 is a protein called Potassium voltage-gated channel subfamily KQT member 2,Potassium voltage-gated channel subfamily KQT member 2.

Mol	Chain	Residues		Atoms					Trace
1	Λ	115	Total	С	Η	Ν	0	S	0
	A	110	1900	611	941	176	167	5	0

There are 37 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
А	1	MET	-	initiating methionine	UNP O43526
A	2	SER	-	expression tag	UNP 043526
A	3	TYR	-	expression tag	UNP O43526
A	4	TYR	-	expression tag	UNP 043526
A	5	HIS	-	expression tag	UNP O43526
A	6	HIS	-	expression tag	UNP O43526
А	7	HIS	-	expression tag	UNP O43526
A	8	HIS	-	expression tag	UNP O43526
A	9	HIS	-	expression tag	UNP O43526
A	10	HIS	-	expression tag	UNP O43526
A	11	ASP	-	expression tag	UNP O43526
A	12	TYR	-	expression tag	UNP O43526
A	13	ASP	-	expression tag	UNP O43526
A	14	ILE	-	expression tag	UNP O43526
A	15	PRO	-	expression tag	UNP O43526
A	16	THR	-	expression tag	UNP O43526
A	17	THR	-	expression tag	UNP O43526
A	18	GLU	-	expression tag	UNP O43526
A	19	ASN	-	expression tag	UNP O43526
A	20	LEU	-	expression tag	UNP O43526
A	21	TYR	-	expression tag	UNP O43526
A	22	PHE	-	expression tag	UNP O43526
A	23	GLN	-	expression tag	UNP O43526
A	24	GLY	-	expression tag	UNP O43526
А	25	ALA	-	expression tag	UNP O43526
А	26	MET	-	expression tag	UNP O43526
A	310	GLY	-	linker	UNP O43526
А	311	ILE	-	linker	UNP O43526
А	312	LEU	-	linker	UNP O43526
А	313	GLY	-	linker	UNP 043526

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Chain	Residue	Modelled	Actual	Comment	Reference
A	314	SER	-	linker	UNP O43526
A	315	GLY	-	linker	UNP O43526
A	373	ARG	-	linker	UNP O43526
A	392	GLY	-	linker	UNP O43526
A	393	LEU	-	linker	UNP O43526
A	548	LEU	-	linker	UNP O43526
A	549	ASP	-	$_{ m linker}$	UNP O43526

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• Molecule 2 is a protein called Calmodulin-1.

Mol	Chain	Residues	Atoms				Trace		
0	В	148	Total	С	Η	Ν	0	S	0
	D	140	2260	714	1094	188	255	9	0

• Molecule 3 is CALCIUM ION (three-letter code: CA) (formula: Ca).

Mol	Chain	Residues	Atoms
2	р	4	Total Ca
კ	В	4	4 4



4 Residue-property plots (i)

4.1 Average score per residue in the NMR ensemble

These plots are provided for all protein, RNA and DNA chains in the entry. The first graphic is the same as shown in the summary in section 1 of this report. The second graphic shows the sequence where residues are colour-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. Stretches of 2 or more consecutive residues without any outliers are shown as green connectors. Residues which are classified as ill-defined in the NMR ensemble, are shown in cyan with an underline colour-coded according to the previous scheme. Residues which were present in the experimental sample, but not modelled in the final structure are shown in grey.

 \bullet Molecule 1: Potassium voltage-gated channel subfamily KQT member 2, Potassium voltage-gated channel subfamily KQT member 2

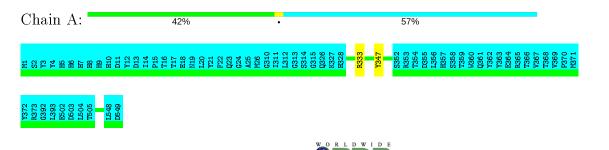
Chain A:	43%	·	57%	
M1 S2 Y4 H5 H7 H3 H3 H10 H10	Y 11 113 114 115 115 115 115 115 115 115 115 115	A25 M26 G310 I311 I311 I315 G315 G315 G315 G315 H328 H328 H328	r355 8355 8355 13554 13555 13555 13555 13556 13556 13556 13556 13563 13563 13563 13563 13563 13563	T366 T366 V367 V369 P370 M371 Y372 R373
(392 E502 E502 E503 1504 1504 1548 D549 D549				
• Molecule 2:	Calmodulin-1			
Chain B:		93%		7% •
MET 122 123 124 124 127 127 127 127 127 127 127 127 127 127	80 K148			

4.2 Scores per residue for each member of the ensemble

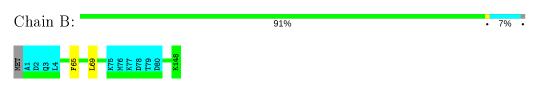
Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1 (medoid)

 \bullet Molecule 1: Potassium voltage-gated channel subfamily KQT member 2, Potassium voltage-gated channel subfamily KQT member 2



• Molecule 2: Calmodulin-1



4.2.2 Score per residue for model 2

 \bullet Molecule 1: Potassium voltage-gated channel subfamily KQT member 2, Potassium voltage-gated channel subfamily KQT member 2

Chain A:	42%	·	57%		
전 22 전 25 전 25 전 25 전 25 전 25 전 25 전 25	D11 712 713 714 714 715 715 715 715 715 715 715 715 715 715	F22 Q23 Q24 Q25 Q24 Q25 Q311 C311 C311 C311 C311 C311 C311 C311	13.28 13.27 13.27 13.55 15.55 15.55 15.55 15.55 15.55 15.55 15.55 15.55 15.55 15.55 15.55	H357 S358 T356 W366 W365 F366 F366 T366 T366 T366 V367 V367 V369	P370 M371
Y372 R373 C392 L393 E502 D503 L504 T504 L548	D549				
• Molecule 2:	Calmodulin-1				
Chain B:		92%		• 7% •	
MET A1 D2 Q3 Q3 L4 K77 K77 D78	179 D80 L116 K148				

4.2.3 Score per residue for model 3

 \bullet Molecule 1: Potassium voltage-gated channel subfamily KQT member 2, Potassium voltage-gated channel subfamily KQT member 2

Chain A:	43%	57%	
M1 22 45 45 46 43 410 410	D11 D13 D13 D13 D13 D13 D13 D13 D13 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2 D2	1311 1312 1312 1312 1313 1313 1314 1325 133555 133555 133555 133555 133555 133555 133555 133555 1335555 1335555 1335555 13355555 1335555 13355555555	1366 7368 7368 7370 7371 7372 7372 6392 1393
1502 1504 1504 1504 1548 1548 1549			
• Molecule 2:	Calmodulin-1		
Chain B:		91%	• 7% •
MET A 1 0 2 0 3 7 12 7 12 7 12 7 12 7 12 7 12 7 12 7 12	K77 1798 1800 1116 1146 1148		



R373

4.2.4 Score per residue for model 4

 \bullet Molecule 1: Potassium voltage-gated channel subfamily KQT member 2, Potassium voltage-gated channel subfamily KQT member 2

Chain A:	43%)	•		57%		_
M1 S2 Y3 H5 H6 H8 H9 H10	D11 712 715 715 717 717	N 19 120 122 123 123 123 123 123 123 123 123 123	M26 G310 I311 L312	6313 S314 G315 Q326 H328 H328	F346 S352 R353 T354 D355 L356 H355 C355	7359 T359 T359 T359 T361 T363 T365 T365	V367 T368 V369 P370 M371 Y372 R373
(3392) 1333 1532 1504 1505 1548 1548 1548							
• Molecule 2:	Calmoduli	n-1					
Chain B:			920	%		• 7	7% •
MET D2 D3 Q3 V7 K7 D78 D78	T79 D80 Y138 K148						

4.2.5 Score per residue for model 5

 \bullet Molecule 1: Potassium voltage-gated channel subfamily KQT member 2, Potassium voltage-gated channel subfamily KQT member 2

Chain A:	43%	·	57%	
M1 S2 Y3 Y3 H3 H3 H3 H10 Y11 Y12	D13 114 115 115 115 117 117 118 118 120 120 120 122 120 122 122 120	A25 M26 G310 C311 C312 C313 C315 C315 C315 C315 C315 C315 C315	F346 F355 F355 F355 F355 F355 F355 F355 F35	Y363 F364 F365 T366 V367 Y369 M371 M371
(3392) 1393 1502 1503 1504 1548 1548 1549				
• Molecule 2: C	almodulin-1			
Chain B:		91%		• 7% •
MET A1 D2 D2 D2 D2 M2 K75 M76 M76 D78 D78 D78 D78 D78 D78	D122 1138 K148			

4.2.6 Score per residue for model 6

 \bullet Molecule 1: Potassium voltage-gated channel subfamily KQT member 2, Potassium voltage-gated channel subfamily KQT member 2

Chain A:	42%	•	57%
0			



M1 822 733 745 745 745 741 7116 7116 7116 7116 7116 7116 7116	1311 L312 G313 G315 G315 G315 H328 F326 F328 F328 F346	2355 7355 7355 7355 7355 7355 7356 7356 7	R365 T366 T366 T368 T368 T368 T368 M371 M371 R372 R373
(392 1504 1504 1504 1549 1549			
• Molecule 2: Calmodulin-1			
Chain B:	91%		• 7% •
MET A1 A1 03 03 14 14 17 17 17 17 17 17 12 12 12 13 13 13 13 14 13 14 13 14 13 14 13 14 14 14 14 14 14 14 14 14 14 14 14 14			

4.2.7Score per residue for model 7

• Molecule 1: Potassium voltage-gated channel subfamily KQT member 2,Potassium voltage-gated channel subfamily KQT member 2

Chain A:	43%		·		57%	
M1 X3 X3 X3 X3 X4 X3 X4 X3 X4 X4 X4 X4 X4 X4 X4 X4 X4 X4 X4 X4 X4	D112 712 713 715 715 813 N19 N19	120 721 722 723 725 725 755	6310 1311 1312 6313 8314 6315	Q326 K327 H328 S352 K353 K353 T354	1355 1355 1355 1355 1359 1359 1350 1350 1350 1350 1350	E364 R365 T366 V367 V367 P370 M371 X372
6392 L393 E502 D503 L504 L548 L548 D549						
• Molecule 2:	Calmodulin	ı-1				
Chain B:			91%			• 7% •
MET A1 C2 C3 C3 F1 F1 C M5 R	K7 5 M7 6 K7 7 D7 8 D7 8 D7 8 D8 0 L1 16	K148				

Score per residue for model 8 4.2.8

• Molecule 1: Potassium voltage-gated channel subfamily KQT member 2, Potassium voltage-gated channel subfamily KQT member 2

Chain A:	43%	•	57%		
1日日 1日日 1日日 1日日 1日 1日 1日 1日 1日 1日 1日 1日	80 111 111 111 111 111 111 111 111 111 1	Y21 F22 G24 G24 A25 M26 G311 L312 L312 L313 C313 S314	6315 6315 6316 6316 7326 7326 7328 7328 7352 7352 7355 7355 7355 7355 7355 7355	H357 S358 T359 H350 W360 V362 Y362 Y363 T366 T366 T366 T366 T366	P370 M371 Y372 R373

• Molecule 2: Calmodulin-1

5 5 8





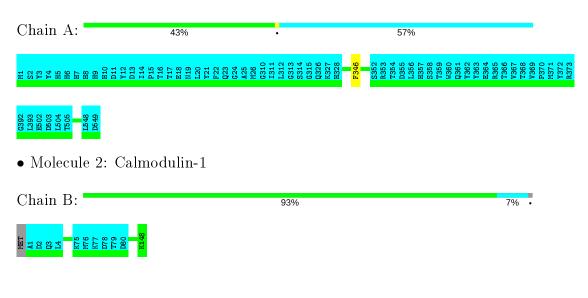
4.2.9 Score per residue for model 9

 \bullet Molecule 1: Potassium voltage-gated channel subfamily KQT member 2, Potassium voltage-gated channel subfamily KQT member 2

Chain A:	43%	·	57%	
M1 22 23 23 23 23 24 24 21 21 21 21	Y12 D13 114 115 115 115 114 113 114 113 114 113 114 113 114 113 114 113 114 113 114 113 114 113 114 114	A25 M26 G310 I311 I311 I311 G315 G315 G315 G315 H326 H328 H328	R355 R355 R355 R355 R355 R355 R355 R355	R366 1366 1366 1368 1368 1368 1368 1368 1
E502 D503 L504 T505 R513 L548 D549 D549				
• Molecule 2: 0	Calmodulin-1			
Chain B:		92%		• 7% •
MET A1 D2 D2 D2 C1 F12 K77 K77 K77	D78 1779 280 K148			

4.2.10 Score per residue for model 10

• Molecule 1: Potassium voltage-gated channel subfamily KQT member 2,Potassium voltage-gated channel subfamily KQT member 2





5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: torsion angle dynamics.

Of the 200 calculated structures, 10 were deposited, based on the following criterion: *structures with the least restraint violations*.

The following table shows the software used for structure solution, optimisation and refinement.

Software name	Classification	Version
ARIA	structure calculation	2.3.1
ARIA	refinement	2.3.1

The following table shows chemical shift validation statistics as aggregates over all chemical shift files. Detailed validation can be found in section 6 of this report.

Chemical shift file(s)	input_cs.cif
Number of chemical shift lists	1
Total number of shifts	2851
Number of shifts mapped to atoms	2851
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	82%

No validations of the models with respect to experimental NMR restraints is performed at this time.

COVALENT-GEOMETRY INFOmissingINFO

5.1 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 each 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 averaged over the ensemble.

Mol	Chain	Non-H	H(model)	H(added)	Clashes
1	А	411	442	442	0±0
2	В	1087	1017	1016	0±0
All	All	15020	14590	14579	2

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 0.

All unique clashes are listed below, sorted by their clash magnitude.



Atom-1	Atom-2	Clash(Å)	Distance(Å)	Moo	dels
Atom-1	Atom-2	Clash(A)	Distance(A)	Worst	Total
2:B:65:PHE:O	2:B:69:LEU:N	0.45	2.45	1	1
1:A:341:GLN:HG3	2:B:116:LEU:HD22	0.41	1.93	2	1

5.2 Torsion angles (i)

5.2.1 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 PDB entries followed by that with respect to all NMR entries. 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	Perce	ntiles
1	А	50/115~(43%)	50 ± 0 (99 $\pm1\%$)	0±0 (1±1%)	0±0 (0±0%)	100	100
2	В	137/149~(92%)	135 ± 2 (99 $\pm1\%$)	$2\pm2~(1\pm1\%)$	0±0 (0±0%)	100	100
All	All	1870/2640~(71%)	1849~(99%)	21 (1%)	0 (0%)	100	100

There are no Ramachandran outliers.

5.2.2 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 PDB entries followed by that with respect to all NMR entries. 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	А	43/102~(42%)	42 ± 1 (97 $\pm1\%$)	$1 \pm 1 (3 \pm 1\%)$	49 91
2	В	117/127~(92%)	$116\pm1 (99\pm1\%)$	$1\pm1 (1\pm1\%)$	84 97
All	All	1600/2290~(70%)	$1581 \ (99\%)$	19 (1%)	72 96

All 10 unique residues with a non-rotameric sidechain are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	А	346	PHE	6
2	В	138	TYR	3
2	В	116	LEU	2
1	А	347	TYR	2

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	J J	1	1 0	
Mol	Chain	\mathbf{Res}	Type	Models (Total)
1	А	513	ARG	1
1	А	526	LYS	1
2	В	122	ASP	1
2	В	53	ASN	1
1	А	333	ARG	1
2	В	135	GLN	1

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5.2.3 RNA (i)

There are no RNA molecules in this entry.

5.3 Non-standard residues in protein, DNA, RNA chains (i)

There are no non-standard protein/DNA/RNA residues in this entry.

5.4 Carbohydrates (i)

There are no carbohydrates in this entry.

5.5 Ligand geometry (i)

Of 4 ligands modelled in this entry, 4 are monoatomic - leaving 0 for Mogul analysis.

5.6 Other polymers (i)

There are no such molecules in this entry.

5.7 Polymer linkage issues (i)

There are no chain breaks in this entry.



6 Chemical shift validation (i)

The completeness of assignment taking into account all chemical shift lists is 82% for the well-defined parts and 77% for the entire structure.

6.1 Chemical shift list 1

File name: input_cs.cif

Chemical shift list name: assigned_chem_shift_list_1

6.1.1 Bookkeeping (i)

The following table shows the results of parsing the chemical shift list and reports the number of nuclei with statistically unusual chemical shifts.

Total number of shifts	2851
Number of shifts mapped to atoms	2851
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	1

6.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	${\bf Correction}\pm{\bf precision},ppm$	Suggested action
$^{13}C_{\alpha}$	252	0.01 ± 0.06	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	234	0.01 ± 0.05	None needed (< 0.5 ppm)
$^{13}C'$	250	0.04 ± 0.06	None needed (< 0.5 ppm)
¹⁵ N	239	0.04 ± 0.26	None needed (< 0.5 ppm)

6.1.3 Completeness of resonance assignments (i)

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 82%, i.e. 1901 atoms were assigned a chemical shift out of a possible 2331. 15 out of 24 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathrm{H}$	$^{13}\mathrm{C}$	15 N
Backbone	919/932~(99%)	367/372~(99%)	373/376 (99%)	$179/184 \ (97\%)$
Sidechain	914/1247~(73%)	559/727~(77%)	355/456~(78%)	0/64~(0%)

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	Total	$^{1}\mathrm{H}$	$^{13}\mathrm{C}$	$^{15}\mathbf{N}$
Aromatic	68/152~(45%)	68/82~(83%)	0/67~(0%)	0/3~(0%)
Overall	1901/2331~(82%)	994/1181 (84%)	728/899~(81%)	$179/251\ (71\%)$

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The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 77%, i.e. 2535 atoms were assigned a chemical shift out of a possible 3273. 21 out of 33 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathrm{H}$	$^{13}\mathrm{C}$	$^{15}\mathbf{N}$
Backbone	1234/1303~(95%)	493/520~(95%)	502/526~(95%)	239/257~(93%)
Sidechain	1207/1682~(72%)	737/983~(75%)	470/618~(76%)	0/81~(0%)
Aromatic	94/288~(33%)	94/153~(61%)	0/120~(0%)	0/15~(0%)
Overall	2535/3273~(77%)	1324/1656~(80%)	972/1264~(77%)	239/353~(68%)

6.1.4 Statistically unusual chemical shifts (i)

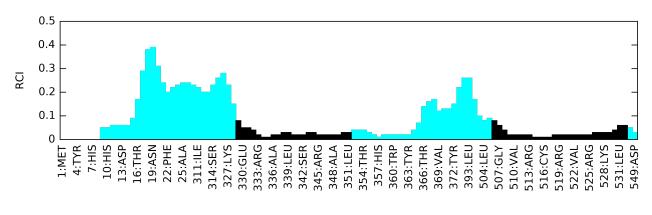
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
2	В	93	ASP	HB2	1.15	4.07 - 1.37	-5.8

6.1.5 Random Coil Index (RCI) plots (

The images below report *random coil index* values for the protein chains in the structure. The height of each bar gives a probability of a given residue to be disordered, as predicted from the available chemical shifts and the amino acid sequence. A value above 0.2 is an indication of significant predicted disorder. The colour of the bar shows whether the residue is in the well-defined core (black) or in the ill-defined residue ranges (cyan), as described in section 2 on ensemble composition.

Random coil index (RCI) for chain A:





Random coil index (RCI) for chain B:

