Full wwPDB NMR Structure Validation Report (i)

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PDB ID	:	6D8T
BMRB ID	:	30463
Title	:	NMR solution structure of tamapin, mutant E25K/K27E
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Deposited on	:	2018-04-26

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 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:

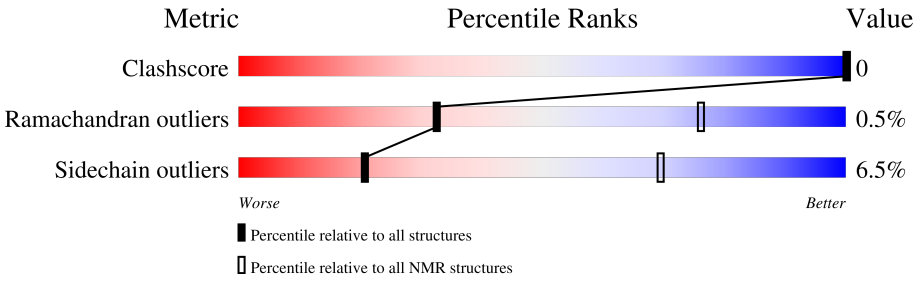
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
BMRB Restraints Analysis	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.33

1 Overall quality at a glance

The following experimental techniques were used to determine the structure:
SOLUTION NMR

The overall completeness of chemical shifts assignment is 50%.

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)	NMR archive (#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	A	31	81% 13% 6%

2 Ensemble composition and analysis (i)

This entry contains 20 models. Model 10 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *lowest energy*.

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:3-A:31 (29)	0.63	10

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. No single-model clusters were found.

Cluster number	Models
1	1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20
2	6, 8, 15

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 476 atoms, of which 239 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Potassium channel toxin alpha-KTx 5.4.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	31	476	146	239	43	42	6	0

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	25	LYS	GLU	engineered mutation	UNP P59869
A	27	GLU	LYS	engineered mutation	UNP P59869

4 Residue-property plots

4.1 Average score per residue in the NMR ensemble

These plots are provided for all protein, RNA, DNA and oligosaccharide 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.

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

Chain A: 

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

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

Chain A: 

4.2.2 Score per residue for model 2

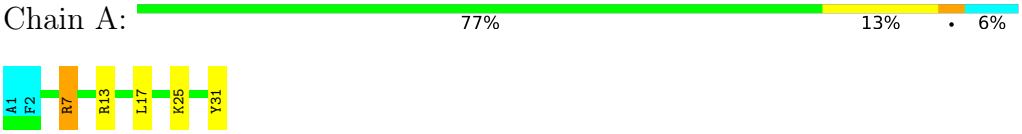
• Molecule 1: Potassium channel toxin alpha-KTx 5.4

Chain A: 

4.2.3 Score per residue for model 3

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

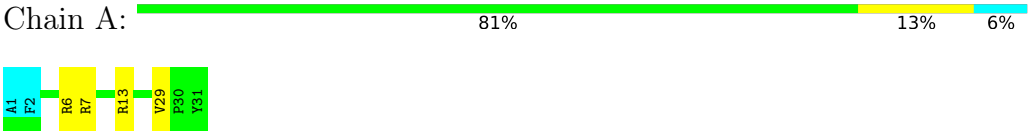
Chain A:



4.2.4 Score per residue for model 4

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

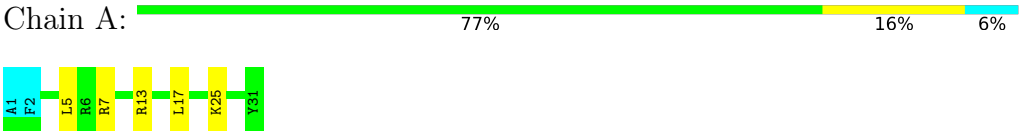
Chain A:



4.2.5 Score per residue for model 5

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

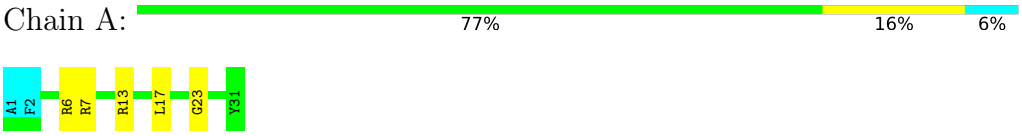
Chain A:



4.2.6 Score per residue for model 6

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

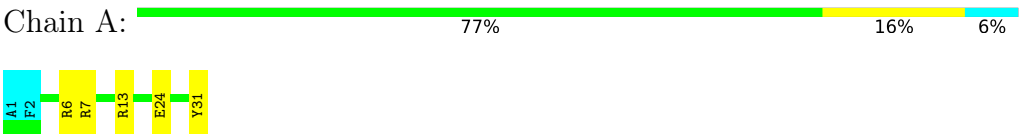
Chain A:



4.2.7 Score per residue for model 7

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

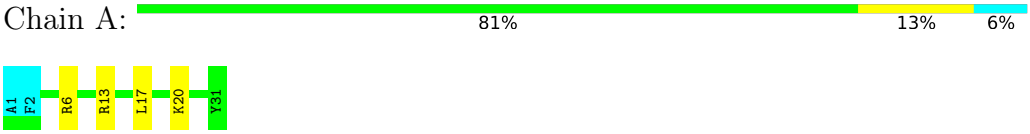
Chain A:



4.2.8 Score per residue for model 8

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

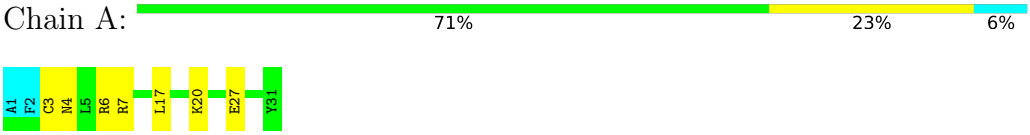
Chain A:



4.2.9 Score per residue for model 9

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

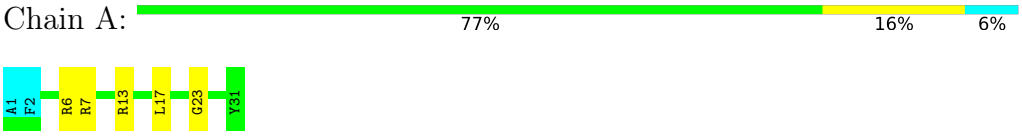
Chain A:



4.2.10 Score per residue for model 10 (medoid)

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

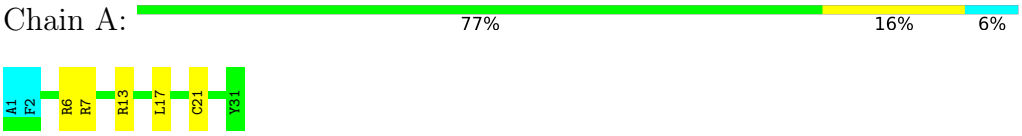
Chain A:



4.2.11 Score per residue for model 11

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

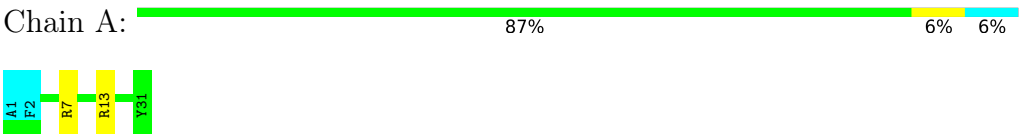
Chain A:



4.2.12 Score per residue for model 12

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

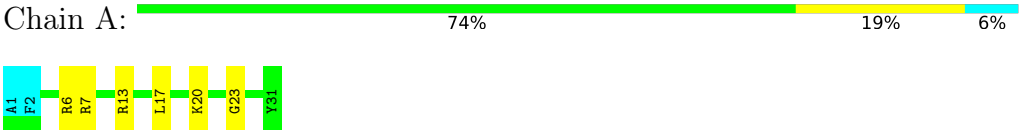
Chain A:



4.2.13 Score per residue for model 13

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

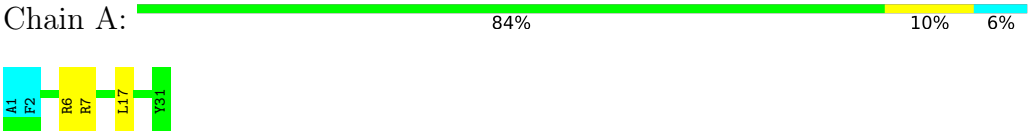
Chain A:



4.2.14 Score per residue for model 14

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

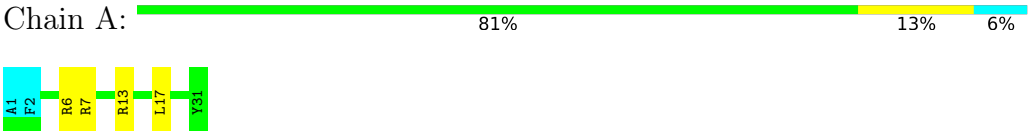
Chain A:



4.2.15 Score per residue for model 15

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

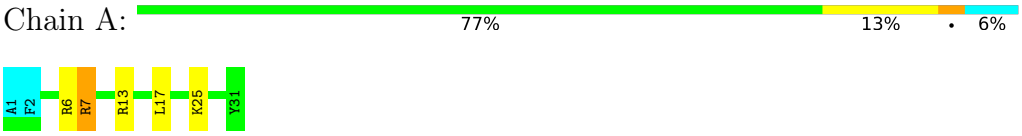
Chain A:



4.2.16 Score per residue for model 16

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

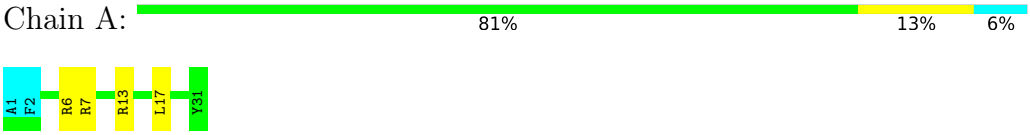
Chain A:



4.2.17 Score per residue for model 17

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

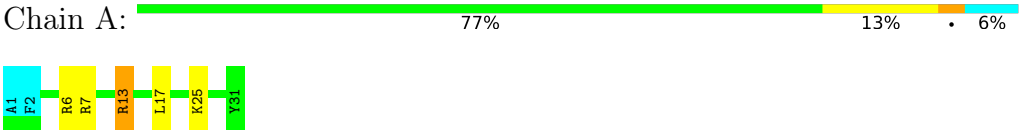
Chain A:



4.2.18 Score per residue for model 18

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

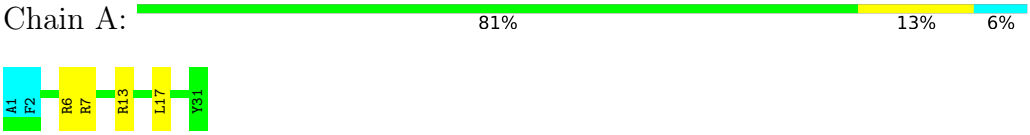
Chain A:



4.2.19 Score per residue for model 19

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

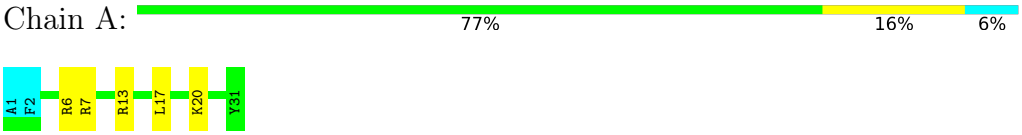
Chain A:



4.2.20 Score per residue for model 20

• Molecule 1: Potassium channel toxin alpha-KTx 5.4

Chain A:



5 Refinement protocol and experimental data overview

The models were refined using the following method: *molecular dynamics*.

Of the 200 calculated structures, 20 were deposited, based on the following criterion: *structures with the lowest energy*.

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

Software name	Classification	Version
Amber	refinement	16
CYANA	structure calculation	2.1

The following table shows chemical shift validation statistics as aggregates over all chemical shift files. Detailed validation can be found in section 7 of this report.

Chemical shift file(s)	working_cs.cif
Number of chemical shift lists	1
Total number of shifts	206
Number of shifts mapped to atoms	206
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	50%

6 Model quality (i)

6.1 Standard geometry (i)

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 (average) 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	0.71±0.01	0±0/222 (0.0± 0.0%)	1.24±0.05	3±1/293 (0.9± 0.3%)
All	All	0.71	0/4440 (0.0%)	1.24	55/5860 (0.9%)

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	Planarity
1	A	0.0±0.0	0.1±0.3
All	All	0	2

There are no bond-length outliers.

All unique angle outliers are listed below. They are sorted according to the Z-score of the worst occurrence in the ensemble.

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
1	A	13	ARG	NE-CZ-NH1	8.89	124.75	120.30	8	18
1	A	7	ARG	NE-CZ-NH1	8.67	124.64	120.30	11	19
1	A	6	ARG	NE-CZ-NH1	8.17	124.39	120.30	20	14
1	A	7	ARG	NE-CZ-NH2	-5.97	117.31	120.30	13	2
1	A	31	TYR	CB-CG-CD2	-5.63	117.62	121.00	7	1
1	A	31	TYR	CB-CG-CD1	-5.04	117.98	121.00	3	1

There are no chirality outliers.

All unique planar outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Group	Models (Total)
1	A	7	ARG	Sidechain	1
1	A	13	ARG	Sidechain	1

6.2 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 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
All	All	4420	4460	4460	-

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 -.

There are no clashes.

6.3 Torsion angles

6.3.1 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 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	Percentiles
1	A	28/31 (90%)	26±1 (92±4%)	2±1 (7±4%)	0±0 (1±1%)	32 76
All	All	560/620 (90%)	516 (92%)	41 (7%)	3 (1%)	32 76

All 1 unique Ramachandran outliers are listed below.

Mol	Chain	Res	Type	Models (Total)
1	A	23	GLY	3

6.3.2 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 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	A	26/27 (96%)	24±1 (93±4%)	2±1 (7±4%)	21 69

Continued on next page...

Continued from previous page...

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
All	All	520/540 (96%)	486 (93%)	34 (7%)	21 69

All 12 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	A	17	LEU	17
1	A	25	LYS	4
1	A	20	LYS	4
1	A	7	ARG	1
1	A	29	VAL	1
1	A	5	LEU	1
1	A	24	GLU	1
1	A	6	ARG	1
1	A	3	CYS	1
1	A	4	ASN	1
1	A	27	GLU	1
1	A	21	CYS	1

6.3.3 RNA

There are no RNA molecules in this entry.

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

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

6.5 Carbohydrates

There are no monosaccharides in this entry.

6.6 Ligand geometry

There are no ligands in this entry.

6.7 Other polymers

There are no such molecules in this entry.

6.8 Polymer linkage issues (i)

There are no chain breaks in this entry.

7 Chemical shift validation

The completeness of assignment taking into account all chemical shift lists is 50% for the well-defined parts and 50% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *depositoe25k_k27e.str*

7.1.1 Bookkeeping

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	206
Number of shifts mapped to atoms	206
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	0

7.1.2 Chemical shift referencing

No chemical shift referencing corrections were calculated (not enough data).

7.1.3 Completeness of resonance assignments

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 50%, i.e. 194 atoms were assigned a chemical shift out of a possible 387. 0 out of 6 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	60/146 (41%)	60/60 (100%)	0/58 (0%)	0/28 (0%)
Sidechain	130/232 (56%)	130/150 (87%)	0/70 (0%)	0/12 (0%)
Aromatic	4/9 (44%)	4/4 (100%)	0/5 (0%)	0/0 (—%)
Overall	194/387 (50%)	194/214 (91%)	0/133 (0%)	0/40 (0%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 50%, i.e. 206 atoms were assigned a chemical shift out of a possible 414. 0 out of 6 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	63/156 (40%)	63/64 (98%)	0/62 (0%)	0/30 (0%)
Sidechain	135/239 (56%)	135/155 (87%)	0/72 (0%)	0/12 (0%)
Aromatic	8/19 (42%)	8/9 (89%)	0/10 (0%)	0/0 (—%)
Overall	206/414 (50%)	206/228 (90%)	0/144 (0%)	0/42 (0%)

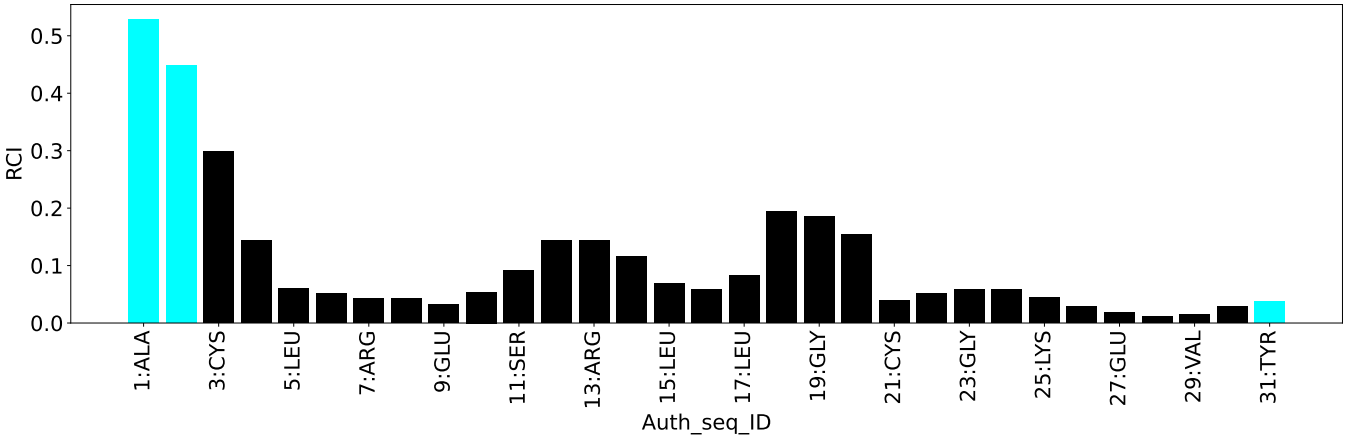
7.1.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

7.1.5 Random Coil Index (RCI) plots (i)

The image below reports *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.If well-defined core and ill-defined regions are not identified then it is shown as gray bars.

Random coil index (RCI) for chain A:



8 NMR restraints analysis

No restraints data found

9 Distance violation analysis (i)

No distance restraints data found

10 Dihedral-angle violation analysis (i)

Dihedral angle analysis failed due to data error in the dihedral angle restraints, possibly missing target value