# Full wwPDB NMR Structure Validation Report (i)

Apr 21, 2024 – 07:59 AM EDT

| | | |
|---|---|---|
| PDB ID | : | 2LKX |
| BMRB ID | : | 18015 |
| Title | : | NMR structure of the homeodomain of Pitx2 in complex with a TAATCC DNA binding site |
| Authors | : | Baird-Titus, J.M.; Doerdelmann, T.; Chaney, B.A.; Clark-Baldwin, K.; Dave, V.; Ma, J. |
| Deposited on | : | 2011-10-21 |

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:

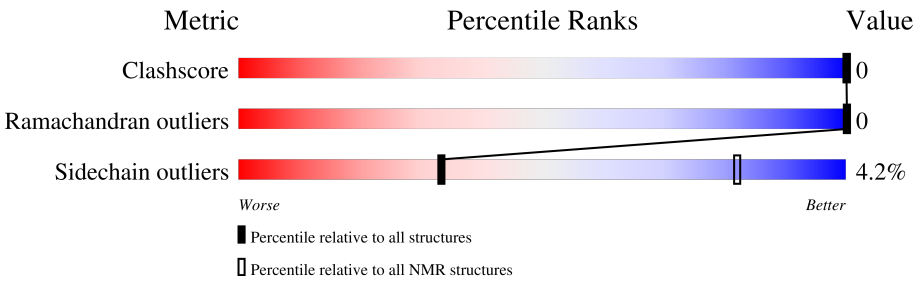
| | | |
|---|---|---|
| MolProbity | : | 4.02b-467 |
| Percentile statistics | : | 20191225.v01 (using entries in the PDB archive December 25th 2019) |
| wwPDB-RCI | : | v_1n_11_5_13_A (Berjanski et al., 2005) |
| PANAV | : | Wang et al. (2010) |
| wwPDB-ShiftChecker | : | v1.2 |
| Ideal geometry (proteins) | : | 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:
*SOLUTION NMR*

The overall completeness of chemical shifts assignment is 46%.

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 | 68 | 57% 6% • 35% |
| 2 | B | 13 | 8% 92% |
| 3 | C | 13 | 8% 92% |

# 2 Ensemble composition and analysis (i)

This entry contains 17 models. Model 8 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *closest to the average*.

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:9-A:52 (44) | 0.46 | 8 |

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 6 single-model clusters were found.

| Cluster number | Models |
|---|---|
| 1 | 2, 4, 8, 12, 13, 14 |
| 2 | 1, 3, 5, 7, 16 |
| Single-model clusters | 6; 9; 10; 11; 15; 17 |

# 3 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 2010 atoms, of which 884 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Pituitary homeobox 3.

| Mol | Chain | Residues | Atoms | | | | | | Trace |
|---|---|---|---|---|---|---|---|---|---|
| | | | Total | C | H | N | O | S | |
| 1 | A | 68 | 1188 | 370 | 589 | 121 | 107 | 1 | 0 |

There are 8 discrepancies between the modelled and reference sequences:

| Chain | Residue | Modelled | Actual | Comment | Reference |
|---|---|---|---|---|---|
| A | -2 | GLY | - | expression tag | UNP O75364 |
| A | -1 | SER | - | expression tag | UNP O75364 |
| A | 61 | GLU | - | expression tag | UNP O75364 |
| A | 62 | PHE | - | expression tag | UNP O75364 |
| A | 63 | ILE | - | expression tag | UNP O75364 |
| A | 64 | VAL | - | expression tag | UNP O75364 |
| A | 65 | THR | - | expression tag | UNP O75364 |
| A | 66 | ASP | - | expression tag | UNP O75364 |

- Molecule 2 is a DNA chain called DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3').

| Mol | Chain | Residues | Atoms | | | | | | Trace |
|---|---|---|---|---|---|---|---|---|---|
| | | | Total | C | H | N | O | P | |
| 2 | B | 13 | 405 | 124 | 148 | 44 | 77 | 12 | 0 |

- Molecule 3 is a DNA chain called DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3').

| Mol | Chain | Residues | Atoms | | | | | | Trace |
|---|---|---|---|---|---|---|---|---|---|
| | | | Total | C | H | N | O | P | |
| 3 | C | 13 | 417 | 128 | 147 | 55 | 75 | 12 | 0 |

# 4 Residue-property plots (i)

## 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: Pituitary homeobox 3

Chain A: 57% 6% • 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 R22 N23 R24 R31 R44 R52 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 92%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 8% 92%

C80 G81 G82 G83 G84 A85 T86 T87 A88 G89 A90 G91 C92

## 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: Pituitary homeobox 3

Chain A: 57% 6% • 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 Q21 R24 R31 R44 R52 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 69% 23%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 8% 77% 15%

C80 G81 G82 G83 G84 A85 T86 T87 A88 G89 A90 G91 C92

### 4.2.2 Score per residue for model 2

• Molecule 1: Pituitary homeobox 3

Chain A: 57% 7% 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 R31 R44 V45 R46 K50 N51 R52 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 69% 23%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 8% 69% 23%

C80 G81 G82 G83 G84 A85 T86 T87 A88 G89 A90 G91 C92

### 4.2.3 Score per residue for model 3

• Molecule 1: Pituitary homeobox 3

Chain A: 54% 9% • 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 R22 N23 R24 D27 R31 T41 R46 R52 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 69% 31%

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 69% 31%

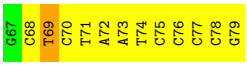
### 4.2.4 Score per residue for model 4

• Molecule 1: Pituitary homeobox 3

Chain A: 59% 6% 35%

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 85% 8%



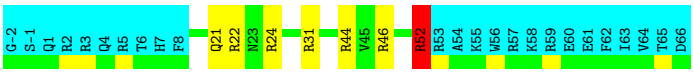

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 23% 46% 31%

### 4.2.5 Score per residue for model 5

• Molecule 1: Pituitary homeobox 3

Chain A: 54% 9% • 35%




• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 85% 8%

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 8% 92%

C80 G81 G82 G83 G84 A85 T86 T87 A88 G89 A90 G91 C92

### 4.2.6 Score per residue for model 6

• Molecule 1: Pituitary homeobox 3

Chain A: 59% 6% 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 R22 R31 R44 R52 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 85% 15%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 15% 85%

C80 G81 G82 G83 G84 A85 T86 T87 A88 G89 A90 G91 C92

### 4.2.7 Score per residue for model 7

• Molecule 1: Pituitary homeobox 3

Chain A: 56% 9% 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 R22 N23 R24 T30 R31 R44 R52 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 77% 15%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 15% 69% 15%

### 4.2.8 Score per residue for model 8 (medoid)

• Molecule 1: Pituitary homeobox 3

Chain A:

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B:

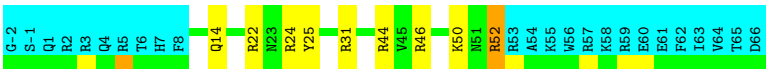
• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C:

### 4.2.9 Score per residue for model 9

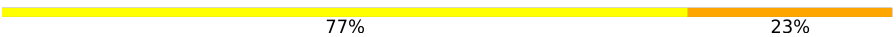
• Molecule 1: Pituitary homeobox 3

Chain A:



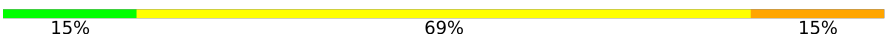

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

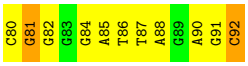
Chain B:



• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C:



### 4.2.10 Score per residue for model 10

- Molecule 1: Pituitary homeobox 3

Chain A: 57% 6% • 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 R24 R31 T41 R44 R52 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

- Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 62% 31%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

- Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 15% 69% 15%

C80 G81 G82 G83 G84 A85 T86 T87 A88 G89 A90 G91 C92

### 4.2.11 Score per residue for model 11

- Molecule 1: Pituitary homeobox 3

Chain A: 57% 6% • 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 R24 R31 R44 V45 R46 R52 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

- Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 69% 23%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

- Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 23% 62% 15%

C80 G81 G82 G83 G84 A85 T86 T87 A88 G89 A90 G91 C92

### 4.2.12 Score per residue for model 12

- Molecule 1: Pituitary homeobox 3

Chain A: 54% 9% • 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 Q21 R22 N23 R24 R31 R44 K50 M51 R52 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 85% 15%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 8% 77% 15%

C80 G81 G82 G83 G84 A85 T86 T87 A88 G89 A90 G91 C92

### 4.2.13 Score per residue for model 13

• Molecule 1: Pituitary homeobox 3

Chain A: 56% • • • 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 T9 R22 N23 R24 R31 R44 R52 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 85% 8%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

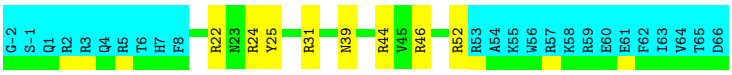
Chain C: 15% 69% 15%

C80 G81 G82 G83 G84 A85 T86 T87 A88 G89 A90 G91 C92

### 4.2.14 Score per residue for model 14

• Molecule 1: Pituitary homeobox 3

Chain A: 53% 12% 35%

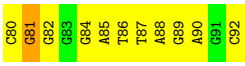



• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 62% 31%

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

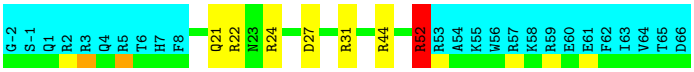
Chain C: 15% 77% 8%




### 4.2.15 Score per residue for model 15

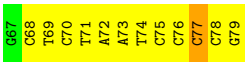
• Molecule 1: Pituitary homeobox 3

Chain A: 54% 9% • 35%




• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 85% 8%




• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 23% 46% 31%

### 4.2.16 Score per residue for model 16

• Molecule 1: Pituitary homeobox 3

Chain A: 57% 7% 35%

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 77% 23%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 23% 69% 8%

C80 G81 G84 A85 T86 T87 A88 G89 A90 G91 C92

### 4.2.17 Score per residue for model 17

• Molecule 1: Pituitary homeobox 3

Chain A: 56% 9% 35%

G-2 S-1 Q1 R2 R3 Q4 R5 T6 H7 F8 R22 N23 R24 Y25 R31 T41 R44 R53 A54 K55 W56 R57 K58 R59 E60 E61 F62 I63 V64 T65 D66

• Molecule 2: DNA (5'-D(*GP*CP*TP*CP*TP*AP*AP*TP*CP*CP*CP*CP*G)-3')

Chain B: 8% 69% 23%

G67 C68 T69 C70 T71 A72 A73 T74 C75 C76 C77 C78 G79

• Molecule 3: DNA (5'-D(*CP*GP*GP*GP*GP*AP*TP*TP*AP*GP*AP*GP*C)-3')

Chain C: 62% 31% 8%

C80 G81 G82 G83 G84 A85 T86 T87 A88 G89 A90 G91 C92

# 5 Refinement protocol and experimental data overview (i)

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

Of the 30 calculated structures, 17 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 |
|---|---|---|
| CYANA | structure solution | |
| Amber | refinement | |

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 | 833 |
| Number of shifts mapped to atoms | 832 |
| Number of unparsed shifts | 0 |
| Number of shifts with mapping errors | 1 |
| Number of shifts with mapping warnings | 0 |
| Assignment completeness (well-defined parts) | 46% |

# 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 | 0.78±0.01 | 0±0/388 ( 0.0± 0.0%) | 1.28±0.06 | 5±1/526 ( 1.0± 0.2%) |
| 2 | B | 1.54±0.02 | 0±0/286 ( 0.0± 0.0%) | 2.36±0.04 | 23±2/438 ( 5.2± 0.4%) |
| 3 | C | 1.57±0.02 | 0±0/304 ( 0.0± 0.0%) | 2.32±0.07 | 21±3/469 ( 4.4± 0.6%) |
| All | All | 1.30 | 0/16626 ( 0.0%) | 2.02 | 830/24361 ( 3.4%) |

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 | 0.6±0.6 |
| 2 | B | 0.0±0.0 | 2.9±1.3 |
| 3 | C | 0.0±0.0 | 3.0±1.4 |
| All | All | 0 | 112 |

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 | 44 | ARG | NE-CZ-NH1 | 10.70 | 125.65 | 120.30 | 15 | 15 |
| 3 | C | 90 | DA | N1-C6-N6 | -10.59 | 112.25 | 118.60 | 2 | 17 |
| 1 | A | 24 | ARG | NE-CZ-NH1 | 10.55 | 125.57 | 120.30 | 5 | 15 |
| 1 | A | 31 | ARG | NE-CZ-NH1 | 10.46 | 125.53 | 120.30 | 11 | 17 |
| 3 | C | 85 | DA | N1-C6-N6 | -10.44 | 112.33 | 118.60 | 7 | 17 |
| 2 | B | 72 | DA | N1-C6-N6 | -10.30 | 112.42 | 118.60 | 1 | 17 |
| 2 | B | 73 | DA | N1-C6-N6 | -10.25 | 112.45 | 118.60 | 8 | 17 |
| 3 | C | 88 | DA | N1-C6-N6 | -10.10 | 112.54 | 118.60 | 4 | 17 |
| 3 | C | 80 | DC | N3-C2-O2 | -9.24 | 115.43 | 121.90 | 5 | 17 |
| 3 | C | 81 | DG | O4'-C1'-N9 | 9.11 | 114.37 | 108.00 | 12 | 14 |
| 1 | A | 52 | ARG | NE-CZ-NH1 | 9.02 | 124.81 | 120.30 | 3 | 14 |

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| Mol | Chain | Res | Type | Atoms | Z | Observed($^o$) | Ideal($^o$) | Models | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Worst | Total |
| 1 | A | 22 | ARG | NE-CZ-NH1 | 9.00 | 124.80 | 120.30 | 14 | 12 |
| 3 | C | 88 | DA | C5-C6-N1 | 8.53 | 121.97 | 117.70 | 7 | 17 |
| 2 | B | 77 | DC | O4'-C1'-N1 | 8.44 | 113.91 | 108.00 | 13 | 6 |
| 1 | A | 31 | ARG | NE-CZ-NH2 | -8.32 | 116.14 | 120.30 | 11 | 8 |
| 2 | B | 73 | DA | C5-C6-N1 | 8.15 | 121.77 | 117.70 | 4 | 17 |
| 3 | C | 90 | DA | C5-C6-N1 | 8.13 | 121.77 | 117.70 | 9 | 17 |
| 2 | B | 72 | DA | C4-C5-C6 | -8.12 | 112.94 | 117.00 | 8 | 17 |
| 2 | B | 72 | DA | C5-C6-N1 | 8.02 | 121.71 | 117.70 | 11 | 17 |
| 3 | C | 90 | DA | C4-C5-C6 | -7.96 | 113.02 | 117.00 | 2 | 17 |
| 3 | C | 90 | DA | O4'-C1'-N9 | 7.91 | 113.53 | 108.00 | 12 | 3 |
| 2 | B | 75 | DC | N3-C2-O2 | -7.87 | 116.39 | 121.90 | 17 | 17 |
| 1 | A | 46 | ARG | NE-CZ-NH1 | 7.83 | 124.21 | 120.30 | 14 | 6 |
| 3 | C | 80 | DC | O4'-C1'-N1 | 7.82 | 113.47 | 108.00 | 5 | 4 |
| 2 | B | 69 | DT | O4'-C1'-N1 | 7.81 | 113.47 | 108.00 | 2 | 1 |
| 2 | B | 68 | DC | N3-C2-O2 | -7.67 | 116.53 | 121.90 | 11 | 17 |
| 2 | B | 74 | DT | O4'-C1'-N1 | 7.64 | 113.35 | 108.00 | 7 | 7 |
| 3 | C | 86 | DT | O4'-C1'-N1 | 7.63 | 113.34 | 108.00 | 9 | 3 |
| 3 | C | 88 | DA | O4'-C1'-N9 | 7.60 | 113.32 | 108.00 | 7 | 2 |
| 3 | C | 92 | DC | N3-C2-O2 | -7.60 | 116.58 | 121.90 | 12 | 17 |
| 2 | B | 79 | DG | O4'-C1'-N9 | 7.59 | 113.31 | 108.00 | 5 | 10 |
| 2 | B | 76 | DC | N3-C2-O2 | -7.55 | 116.61 | 121.90 | 13 | 17 |
| 3 | C | 85 | DA | C5-C6-N1 | 7.55 | 121.47 | 117.70 | 10 | 17 |
| 3 | C | 86 | DT | C6-C5-C7 | -7.46 | 118.43 | 122.90 | 13 | 17 |
| 3 | C | 88 | DA | C4-C5-C6 | -7.43 | 113.28 | 117.00 | 9 | 17 |
| 3 | C | 85 | DA | C4-C5-C6 | -7.38 | 113.31 | 117.00 | 7 | 17 |
| 2 | B | 78 | DC | N3-C2-O2 | -7.25 | 116.82 | 121.90 | 3 | 17 |
| 2 | B | 74 | DT | C6-C5-C7 | -7.24 | 118.56 | 122.90 | 11 | 16 |
| 2 | B | 73 | DA | C4-C5-C6 | -7.11 | 113.45 | 117.00 | 8 | 17 |
| 2 | B | 70 | DC | N3-C2-O2 | -7.06 | 116.96 | 121.90 | 2 | 17 |
| 2 | B | 71 | DT | C6-C5-C7 | -7.00 | 118.70 | 122.90 | 1 | 17 |
| 3 | C | 92 | DC | O4'-C1'-N1 | 6.98 | 112.89 | 108.00 | 4 | 2 |
| 3 | C | 83 | DG | O4'-C1'-N9 | 6.96 | 112.87 | 108.00 | 7 | 4 |
| 3 | C | 82 | DG | O4'-C1'-N9 | 6.86 | 112.80 | 108.00 | 15 | 1 |
| 2 | B | 71 | DT | O4'-C1'-N1 | 6.84 | 112.79 | 108.00 | 14 | 11 |
| 3 | C | 89 | DG | O4'-C1'-N9 | 6.74 | 112.72 | 108.00 | 5 | 5 |
| 2 | B | 77 | DC | N3-C2-O2 | -6.73 | 117.19 | 121.90 | 9 | 17 |
| 3 | C | 87 | DT | C6-C5-C7 | -6.69 | 118.89 | 122.90 | 17 | 17 |
| 2 | B | 71 | DT | N3-C2-O2 | -6.52 | 118.39 | 122.30 | 7 | 11 |
| 2 | B | 69 | DT | C6-C5-C7 | -6.45 | 119.03 | 122.90 | 5 | 17 |
| 3 | C | 84 | DG | P-O3'-C3' | 6.42 | 127.40 | 119.70 | 4 | 3 |
| 2 | B | 76 | DC | O4'-C1'-N1 | 6.32 | 112.43 | 108.00 | 15 | 1 |

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| Mol | Chain | Res | Type | Atoms | Z | Observed($^o$) | Ideal($^o$) | Models | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Worst | Total |
| 3 | C | 89 | DG | N3-C2-N2 | -6.24 | 115.54 | 119.90 | 5 | 4 |
| 3 | C | 88 | DA | O4'-C4'-C3' | 6.22 | 109.73 | 106.00 | 8 | 3 |
| 3 | C | 80 | DC | N1-C2-O2 | 6.20 | 122.62 | 118.90 | 5 | 9 |
| 3 | C | 85 | DA | O4'-C4'-C3' | 6.13 | 109.68 | 106.00 | 14 | 1 |
| 2 | B | 69 | DT | O4'-C4'-C3' | 6.13 | 109.68 | 106.00 | 1 | 2 |
| 3 | C | 92 | DC | N1-C2-O2 | 6.11 | 122.57 | 118.90 | 5 | 10 |
| 3 | C | 83 | DG | C1'-O4'-C4' | -6.07 | 104.03 | 110.10 | 5 | 1 |
| 2 | B | 68 | DC | O4'-C4'-C3' | 6.06 | 109.64 | 106.00 | 5 | 2 |
| 3 | C | 84 | DG | N1-C6-O6 | -6.04 | 116.28 | 119.90 | 14 | 11 |
| 2 | B | 75 | DC | N3-C4-C5 | 6.00 | 124.30 | 121.90 | 14 | 4 |
| 3 | C | 87 | DT | P-O3'-C3' | 5.97 | 126.86 | 119.70 | 1 | 1 |
| 2 | B | 79 | DG | N1-C6-O6 | -5.94 | 116.33 | 119.90 | 2 | 6 |
| 2 | B | 73 | DA | O4'-C4'-C3' | 5.93 | 109.56 | 106.00 | 16 | 6 |
| 3 | C | 87 | DT | N3-C2-O2 | -5.92 | 118.75 | 122.30 | 1 | 9 |
| 2 | B | 68 | DC | N1-C2-O2 | 5.87 | 122.42 | 118.90 | 17 | 13 |
| 3 | C | 81 | DG | N1-C6-O6 | -5.86 | 116.39 | 119.90 | 1 | 6 |
| 2 | B | 75 | DC | O4'-C4'-C3' | 5.86 | 109.52 | 106.00 | 10 | 1 |
| 2 | B | 75 | DC | N1-C2-O2 | 5.83 | 122.40 | 118.90 | 14 | 12 |
| 3 | C | 91 | DG | N1-C6-O6 | -5.79 | 116.43 | 119.90 | 3 | 5 |
| 2 | B | 75 | DC | O4'-C1'-N1 | 5.67 | 111.97 | 108.00 | 6 | 1 |
| 2 | B | 69 | DT | N3-C2-O2 | -5.66 | 118.90 | 122.30 | 16 | 2 |
| 1 | A | 44 | ARG | CD-NE-CZ | 5.65 | 131.51 | 123.60 | 10 | 1 |
| 1 | A | 24 | ARG | NE-CZ-NH2 | -5.63 | 117.49 | 120.30 | 15 | 1 |
| 2 | B | 74 | DT | N3-C2-O2 | -5.63 | 118.92 | 122.30 | 14 | 5 |
| 3 | C | 80 | DC | N3-C4-C5 | 5.61 | 124.15 | 121.90 | 17 | 1 |
| 2 | B | 78 | DC | N1-C2-O2 | 5.50 | 122.20 | 118.90 | 3 | 2 |
| 3 | C | 90 | DA | C1'-O4'-C4' | -5.50 | 104.60 | 110.10 | 8 | 1 |
| 2 | B | 76 | DC | N1-C2-O2 | 5.43 | 122.16 | 118.90 | 13 | 7 |
| 2 | B | 77 | DC | C1'-O4'-C4' | -5.42 | 104.68 | 110.10 | 16 | 1 |
| 3 | C | 80 | DC | O4'-C1'-C2' | -5.40 | 101.58 | 105.90 | 5 | 1 |
| 3 | C | 86 | DT | C5-C6-N1 | -5.39 | 120.47 | 123.70 | 17 | 2 |
| 3 | C | 92 | DC | N3-C4-C5 | 5.38 | 124.05 | 121.90 | 9 | 2 |
| 3 | C | 82 | DG | N1-C6-O6 | -5.38 | 116.67 | 119.90 | 17 | 2 |
| 2 | B | 78 | DC | N3-C4-C5 | 5.37 | 124.05 | 121.90 | 3 | 4 |
| 3 | C | 86 | DT | N3-C2-O2 | -5.36 | 119.08 | 122.30 | 2 | 3 |
| 2 | B | 77 | DC | N1-C2-O2 | 5.36 | 122.11 | 118.90 | 12 | 1 |
| 2 | B | 76 | DC | C1'-O4'-C4' | -5.35 | 104.75 | 110.10 | 6 | 1 |
| 3 | C | 84 | DG | O4'-C4'-C3' | 5.32 | 109.19 | 106.00 | 16 | 3 |
| 2 | B | 68 | DC | N3-C4-C5 | 5.31 | 124.02 | 121.90 | 8 | 1 |
| 2 | B | 70 | DC | O4'-C1'-N1 | 5.29 | 111.70 | 108.00 | 15 | 1 |
| 3 | C | 90 | DA | C6-C5-N7 | 5.26 | 135.98 | 132.30 | 2 | 2 |

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| Mol | Chain | Res | Type | Atoms | Z | Observed($^o$) | Ideal($^o$) | Models | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Worst | Total |
| 2 | B | 71 | DT | C5-C6-N1 | -5.25 | 120.55 | 123.70 | 5 | 2 |
| 2 | B | 71 | DT | N1-C2-N3 | 5.25 | 117.75 | 114.60 | 7 | 1 |
| 2 | B | 70 | DC | N1-C2-O2 | 5.22 | 122.03 | 118.90 | 7 | 5 |
| 2 | B | 67 | DG | N1-C6-O6 | -5.21 | 116.77 | 119.90 | 9 | 2 |
| 2 | B | 73 | DA | C6-C5-N7 | 5.21 | 135.94 | 132.30 | 2 | 1 |
| 3 | C | 89 | DG | N1-C6-O6 | -5.20 | 116.78 | 119.90 | 14 | 4 |
| 3 | C | 91 | DG | O4'-C4'-C3' | 5.18 | 109.11 | 106.00 | 7 | 1 |
| 3 | C | 92 | DC | O4'-C1'-C2' | -5.17 | 101.76 | 105.90 | 4 | 1 |
| 2 | B | 77 | DC | O4'-C4'-C3' | 5.16 | 109.10 | 106.00 | 2 | 1 |
| 3 | C | 91 | DG | O4'-C1'-N9 | 5.16 | 111.61 | 108.00 | 3 | 1 |
| 3 | C | 90 | DA | O4'-C4'-C3' | 5.14 | 109.08 | 106.00 | 10 | 1 |
| 3 | C | 87 | DT | C5-C6-N1 | -5.13 | 120.62 | 123.70 | 10 | 2 |
| 3 | C | 83 | DG | N1-C6-O6 | -5.13 | 116.82 | 119.90 | 3 | 2 |
| 3 | C | 87 | DT | O4'-C4'-C3' | 5.12 | 109.07 | 106.00 | 15 | 1 |
| 2 | B | 72 | DA | C6-C5-N7 | 5.07 | 135.85 | 132.30 | 1 | 1 |
| 2 | B | 77 | DC | N3-C4-C5 | 5.04 | 123.92 | 121.90 | 16 | 1 |
| 2 | B | 69 | DT | C5-C6-N1 | -5.02 | 120.69 | 123.70 | 10 | 1 |
| 2 | B | 74 | DT | C5-C6-N1 | -5.00 | 120.70 | 123.70 | 16 | 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) |
|---|---|---|---|---|---|
| 2 | B | 69 | DT | Sidechain | 8 |
| 3 | C | 81 | DG | Sidechain | 8 |
| 3 | C | 82 | DG | Sidechain | 8 |
| 3 | C | 83 | DG | Sidechain | 8 |
| 2 | B | 72 | DA | Sidechain | 6 |
| 3 | C | 89 | DG | Sidechain | 6 |
| 1 | A | 52 | ARG | Sidechain | 6 |
| 2 | B | 75 | DC | Sidechain | 6 |
| 2 | B | 78 | DC | Sidechain | 6 |
| 2 | B | 79 | DG | Sidechain | 5 |
| 3 | C | 85 | DA | Sidechain | 5 |
| 2 | B | 71 | DT | Sidechain | 4 |
| 2 | B | 77 | DC | Sidechain | 4 |
| 3 | C | 86 | DT | Sidechain | 4 |
| 3 | C | 87 | DT | Sidechain | 4 |
| 2 | B | 67 | DG | Sidechain | 4 |
| 3 | C | 88 | DA | Sidechain | 4 |

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| Mol | Chain | Res | Type | Group | Models (Total) |
|---|---|---|---|---|---|
| 2 | B | 74 | DT | Sidechain | 3 |
| 1 | A | 25 | TYR | Sidechain | 3 |
| 3 | C | 90 | DA | Sidechain | 2 |
| 1 | A | 44 | ARG | Sidechain | 2 |
| 2 | B | 76 | DC | Sidechain | 2 |
| 3 | C | 92 | DC | Sidechain | 2 |
| 2 | B | 68 | DC | Sidechain | 1 |
| 2 | B | 73 | DA | Sidechain | 1 |

## 6.2 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 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 |
|---|---|---|---|---|---|
| 3 | C | 270 | 147 | 147 | 0±0 |
| All | All | 15419 | 11288 | 11288 | 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 0.

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

| Atom-1 | Atom-2 | Clash(Å) | Distance(Å) | Models | |
|---|---|---|---|---|---|
| | | | | Worst | Total |
| 3:C:88:DA:H1' | 3:C:89:DG:C8 | 0.41 | 2.50 | 17 | 1 |

## 6.3 Torsion angles (i)

### 6.3.1 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 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 | 44/68 (65%) | 43±1 (98±2%) | 1±1 (2±2%) | 0±0 (0±0%) | 100 | 100 |
| All | All | 748/1156 (65%) | 734 (98%) | 14 (2%) | 0 (0%) | 100 | 100 |

There are no Ramachandran outliers.

### 6.3.2 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 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 | 41/63 (65%) | 39±1 (96±2%) | 2±1 (4±2%) | 33 82 |
| All | All | 697/1071 (65%) | 668 (96%) | 29 (4%) | 33 82 |

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 | A | 52 | ARG | 12 |
| 1 | A | 21 | GLN | 4 |
| 1 | A | 50 | LYS | 3 |
| 1 | A | 41 | THR | 3 |
| 1 | A | 27 | ASP | 2 |
| 1 | A | 30 | THR | 1 |
| 1 | A | 14 | GLN | 1 |
| 1 | A | 9 | THR | 1 |
| 1 | A | 24 | ARG | 1 |
| 1 | A | 39 | ASN | 1 |

### 6.3.3 RNA (i)

There are no RNA molecules in this entry.

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

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

## 6.5 Carbohydrates (i)

There are no monosaccharides in this entry.

## 6.6 Ligand geometry (i)

There are no ligands in this entry.

## 6.7 Other polymers (i)

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 (i)

The completeness of assignment taking into account all chemical shift lists is 46% for the well-defined parts and 53% for the entire structure.

## 7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *assigned_chem_shift_list_1*

### 7.1.1 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 | 833 |
| Number of shifts mapped to atoms | 832 |
| Number of unparsed shifts | 0 |
| Number of shifts with mapping errors | 1 |
| Number of shifts with mapping warnings | 0 |
| Number of shift outliers (ShiftChecker) | 14 |

The following assigned chemical shifts were not mapped to the molecules present in the coordinate file.

- No matching atom found in the structure. All 1 occurrences are reported below.

| List ID | Chain | Res | Type | Atom | Shift Data | | |
|---|---|---|---|---|---|---|---|
| | | | | | Value | Uncertainty | Ambiguity |
| 1 | A | 7 | HIS | HD1 | 8.744 | 0.020 | 1 |

### 7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

| Nucleus | # values | Correction ± precision, *ppm* | Suggested action |
|---|---|---|---|
| $^{13}C_{\alpha}$ | 61 | -0.55 ± 0.21 | Should be checked |
| $^{13}C_{\beta}$ | 59 | 0.12 ± 0.11 | None needed (< 0.5 ppm) |
| $^{13}C'$ | 0 | — | None (insufficient data) |
| $^{15}N$ | 65 | -0.42 ± 0.43 | None needed (< 0.5 ppm) |

### 7.1.3 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 46%, i.e. 540 atoms were assigned a chemical shift out of a possible 1174. 0 out of 6 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

| | Total | $^{1}$H | $^{13}$C | $^{15}$N |
|---|---|---|---|---|
| Backbone | 172/218 (79%) | 87/87 (100%) | 42/88 (48%) | 43/43 (100%) |
| Sidechain | 322/385 (84%) | 220/245 (90%) | 89/114 (78%) | 13/26 (50%) |
| Aromatic | 46/53 (87%) | 26/26 (100%) | 18/25 (72%) | 2/2 (100%) |
| Sugar | 0/312 (0%) | 0/182 (0%) | 0/130 (0%) | 0/0 (—%) |
| Base | 0/206 (0%) | 0/128 (0%) | 0/44 (0%) | 0/34 (0%) |
| Overall | 540/1174 (46%) | 333/668 (50%) | 149/401 (37%) | 58/105 (55%) |

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 53%, i.e. 827 atoms were assigned a chemical shift out of a possible 1560. 0 out of 7 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

| | Total | $^{1}$H | $^{13}$C | $^{15}$N |
|---|---|---|---|---|
| Backbone | 258/339 (76%) | 132/136 (97%) | 61/136 (45%) | 65/67 (97%) |
| Sidechain | 490/611 (80%) | 336/386 (87%) | 133/177 (75%) | 21/48 (44%) |
| Aromatic | 79/92 (86%) | 45/46 (98%) | 31/42 (74%) | 3/4 (75%) |
| Sugar | 0/312 (0%) | 0/182 (0%) | 0/130 (0%) | 0/0 (—%) |
| Base | 0/206 (0%) | 0/128 (0%) | 0/44 (0%) | 0/34 (0%) |
| Overall | 827/1560 (53%) | 513/878 (58%) | 225/529 (43%) | 89/153 (58%) |

### 7.1.4 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.

| List Id | Chain | Res | Type | Atom | Shift, *ppm* | Expected range, *ppm* | Z-score |
|---|---|---|---|---|---|---|---|
| 1 | A | 9 | THR | HG1 | 5.83 | 0.08 – 2.19 | 22.2 |
| 1 | A | 19 | THR | HG1 | 5.36 | 0.08 – 2.19 | 20.0 |
| 1 | A | 38 | THR | HG1 | 4.08 | 0.08 – 2.19 | 14.0 |
| 1 | A | 52 | ARG | HB2 | -0.51 | 0.52 – 3.08 | -9.0 |
| 1 | A | 52 | ARG | HG3 | -0.60 | 0.15 – 2.94 | -7.7 |
| 1 | A | 16 | LEU | HB3 | -1.20 | -0.26 – 3.31 | -7.7 |
| 1 | A | 52 | ARG | HG2 | -0.21 | 0.26 – 2.87 | -6.8 |
| 1 | A | 24 | ARG | HG3 | -0.29 | 0.15 – 2.94 | -6.6 |
| 1 | A | 26 | PRO | HG3 | -0.12 | 0.33 – 3.48 | -6.4 |
| 1 | A | 24 | ARG | HD2 | 1.71 | 1.97 – 4.26 | -6.2 |

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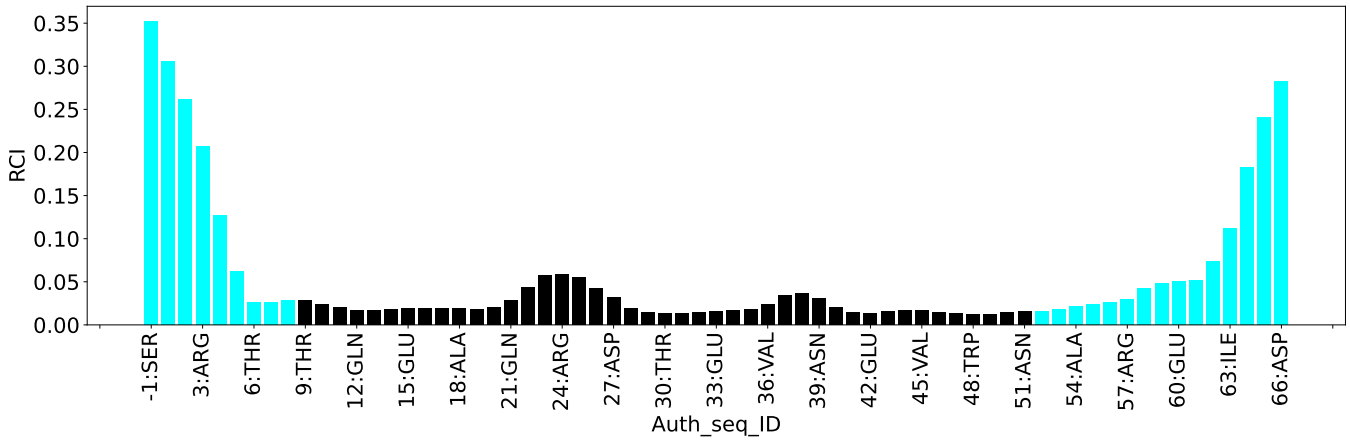
| List Id | Chain | Res | Type | Atom | Shift, *ppm* | Expected range, *ppm* | Z-score |
|---|---|---|---|---|---|---|---|
| 1 | A | 24 | ARG | HD3 | 1.63 | 1.81 – 4.39 | -5.7 |
| 1 | A | 16 | LEU | HD11 | -0.80 | -0.61 – 2.12 | -5.7 |
| 1 | A | 16 | LEU | HD12 | -0.80 | -0.61 – 2.12 | -5.7 |
| 1 | A | 16 | LEU | HD13 | -0.80 | -0.61 – 2.12 | -5.7 |

### 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: