



# Full wwPDB X-ray Structure Validation Report

Mar 1, 2014 – 01:05 AM GMT

PDB ID : 1JB4  
Title : CRYSTAL STRUCTURE OF NTF2 M102E MUTANT  
Authors : Chaillan-Huntington, C.; Butler, P.J.; Huntington, J.A.; Akin, D.; Feldherr, C.; Stewart, M.  
Deposited on : 2001-06-01  
Resolution : 2.23 Å(reported)

This is a full wwPDB validation report for a publicly released PDB entry.  
We welcome your comments at [validation@mail.wwpdb.org](mailto:validation@mail.wwpdb.org)  
A user guide is available at <http://wwpdb.org/ValidationPDFNotes.html>

---

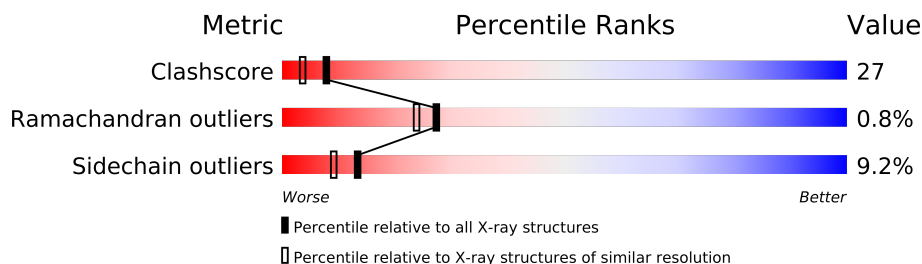
The following versions of software and data (see [references](#)) were used in the production of this report:

MolProbity : 4.02b-467  
Mogul : 1.15 2013  
Xtriage (Phenix) : **NOT EXECUTED**  
EDS : **NOT EXECUTED**  
Percentile statistics : 21963  
Ideal geometry (proteins) : Engh & Huber (2001)  
Ideal geometry (DNA, RNA) : Parkinson et. al. (1996)  
Validation Pipeline (wwPDB-VP) : stable22683

# 1 Overall quality at a glance

The reported resolution of this entry is 2.23 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<br>(#Entries) | Similar resolution<br>(#Entries, resolution range(Å)) |
|-----------------------|-----------------------------|---|
| Clashscore            | 79885                       | 1317 (2.26-2.22)                                      |
| Ramachandran outliers | 78287                       | 1282 (2.26-2.22)                                      |
| Sidechain outliers    | 78261                       | 1282 (2.26-2.22)                                      |

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  $\geq 3$ , 2, 1 and 0 types of geometric quality criteria. The upper red bar (where present) indicates the fraction of residues that have poor fit to the electron density.

Note EDS was not executed.

| Mol | Chain | Length | Quality of chain |
|-----|-------|--------|------------------|
| 1   | A     | 127    |                  |
| 1   | B     | 127    |                  |

## 2 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 2169 atoms, of which 0 are hydrogen and 0 are deuterium.

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 NUCLEAR TRANSPORT FACTOR 2.

| Mol | Chain | Residues | Atoms |     |     |     |   | ZeroOcc | AltConf | Trace |
|-----|-------|----------|-------|-----|-----|-----|---|---------|---------|-------|
| 1   | A     | 123      | Total | C   | N   | O   | S | 5       | 0       | 0     |
|     |       |          | 993   | 636 | 166 | 185 | 6 |         |         |       |
| 1   | B     | 123      | Total | C   | N   | O   | S | 6       | 0       | 0     |
|     |       |          | 993   | 636 | 166 | 185 | 6 |         |         |       |

There are 4 discrepancies between the modelled and reference sequences:

| Chain | Residue | Modelled | Actual | Comment    | Reference  |
|-------|---------|----------|--------|------------|------------|
| A     | 16      | ASN      | GLN    | CONFLICT   | UNP P61972 |
| A     | 102     | GLU      | MET    | ENGINEERED | UNP P61972 |
| B     | 16      | ASN      | GLN    | CONFLICT   | UNP P61972 |
| B     | 102     | GLU      | MET    | ENGINEERED | UNP P61972 |

- Molecule 2 is water.

| Mol | Chain | Residues | Atoms |     | ZeroOcc | AltConf |
|-----|-------|----------|-------|-----|---------|---------|
| 2   | A     | 83       | Total | O   | 0       | 0       |
|     |       |          | 83    | 83  |         |         |
| 2   | B     | 100      | Total | O   | 0       | 0       |
|     |       |          | 100   | 100 |         |         |



## 4 Data and refinement statistics

Xtriage (Phenix) and EDS were not executed - this section will therefore be incomplete.

| Property   | Value   | Source    |
|--|---|-----------|
| Space group  | P 1 21 1                                      | Depositor |
| Cell constants<br>a, b, c, $\alpha$ , $\beta$ , $\gamma$ | 35.15Å 79.07Å 41.65Å<br>90.00° 104.66° 90.00° | Depositor |
| Resolution (Å)   | 30.00 – 2.23                                  | Depositor |
| % Data completeness<br>(in resolution range)             | (Not available) (30.00-2.23)                  | Depositor |
| $R_{merge}$  | 0.09  | Depositor |
| $R_{sym}$  | (Not available)                               | Depositor |
| Refinement program                                       | CNS 1.0                                       | Depositor |
| R, $R_{free}$  | 0.204 , 0.241                                 | Depositor |
| Estimated twinning fraction                              | No twinning to report.                        | Xtriage   |
| Total number of atoms                                    | 2169  | wwPDB-VP  |
| Average B, all atoms (Å <sup>2</sup> )                   | 30.0  | wwPDB-VP  |

## 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     | 0.46         | 0/1019      | 1.01        | 5/1382 (0.4%) |
| 1   | B     | 0.42         | 0/1019      | 0.65        | 0/1382        |
| All | All   | 0.44         | 0/2038      | 0.85        | 5/2764 (0.2%) |

There are no bond length outliers.

All (5) bond angle outliers are listed below:

| Mol | Chain | Res | Type | Atoms   | Z      | Observed(°) | Ideal(°) |
|-----|-------|-----|------|---------|--------|-------------|----------|
| 1   | A     | 94  | ASP  | C-N-CD  | -20.17 | 76.22       | 120.60   |
| 1   | A     | 94  | ASP  | C-N-CA  | 13.73  | 179.67      | 122.00   |
| 1   | A     | 94  | ASP  | N-CA-C  | 8.73   | 134.57      | 111.00   |
| 1   | A     | 95  | PRO  | N-CA-C  | -5.86  | 96.86       | 112.10   |
| 1   | A     | 95  | PRO  | CA-N-CD | -5.58  | 103.68      | 111.50   |

There are no chirality outliers.

There are no planarity outliers.

### 5.2 Close contacts

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 hydrogens added by MolProbity. The Clashes column lists the number of clashes within the asymmetric unit, and the number in parentheses is this value normalized per 1000 atoms of the molecule in the chain. The Symm-Clashes column gives symmetry related clashes, in the same way as for the Clashes column.

| Mol | Chain | Non-H | H(model) | H(added) | Clashes | Symm-Clashes |
|-----|-------|-------|----------|----------|---------|--------------|
| 1   | A     | 993   | 0        | 953      | 62      | 0            |
| 1   | B     | 993   | 0        | 953      | 49      | 0            |
| 2   | A     | 83    | 0        | 0        | 11      | 0            |
| 2   | B     | 100   | 0        | 0        | 8       | 1            |
| All | All   | 2169  | 0        | 1906     | 105     | 1            |

Clashscore is defined as the number of clashes calculated for the entry per 1000 atoms (including hydrogens) of the entry. The overall clashscore for this entry is 27.

All (105) close contacts within the same asymmetric unit are listed below.

| Atom-1           | Atom-2          | Distance(Å) | Clash(Å) |
|------------------|-----------------|-------------|----------|
| 1:A:68:ILE:HD11  | 1:A:85:VAL:HG13 | 1.23        | 1.09     |
| 1:A:96:ILE:CD1   | 1:A:96:ILE:H    | 1.64        | 1.08     |
| 1:A:68:ILE:HD11  | 1:A:85:VAL:CG1  | 1.85        | 1.06     |
| 1:A:16:ASN:HD21  | 1:A:20:GLN:NE2  | 1.51        | 1.06     |
| 1:A:96:ILE:H     | 1:A:96:ILE:HD12 | 0.92        | 1.06     |
| 1:A:96:ILE:N     | 1:A:96:ILE:HD12 | 1.77        | 0.99     |
| 1:A:68:ILE:CD1   | 1:A:85:VAL:HG13 | 1.92        | 0.99     |
| 1:A:104:LEU:HB3  | 2:A:204:HOH:O   | 1.66        | 0.95     |
| 1:A:16:ASN:HD21  | 1:A:20:GLN:HE21 | 1.10        | 0.92     |
| 1:A:16:ASN:HD22  | 1:A:16:ASN:C    | 1.77        | 0.87     |
| 1:B:66:HIS:CD2   | 1:B:89:LEU:HD13 | 2.09        | 0.87     |
| 1:A:107:ASN:HD21 | 1:A:110:ASP:HA  | 1.39        | 0.85     |
| 1:A:115:THR:OG1  | 2:A:204:HOH:O   | 1.96        | 0.82     |
| 1:A:124:HIS:ND1  | 2:A:210:HOH:O   | 2.13        | 0.80     |
| 1:B:4:LYS:HB3    | 1:B:5:PRO:HD3   | 1.63        | 0.79     |
| 1:B:80:CYS:C     | 1:B:81:ILE:HD12 | 2.04        | 0.78     |
| 1:B:124:HIS:CE1  | 1:B:125:ASN:O   | 2.38        | 0.76     |
| 1:A:124:HIS:HB3  | 1:B:122:ALA:HB1 | 1.68        | 0.76     |
| 1:B:124:HIS:ND1  | 1:B:125:ASN:O   | 2.19        | 0.74     |
| 1:A:16:ASN:ND2   | 1:A:20:GLN:NE2  | 2.34        | 0.72     |
| 1:B:124:HIS:ND1  | 1:B:125:ASN:N   | 2.38        | 0.72     |
| 1:A:29:LEU:O     | 1:A:32:ILE:HG13 | 1.90        | 0.71     |
| 2:A:210:HOH:O    | 1:B:100:HIS:NE2 | 2.19        | 0.71     |
| 1:A:20:GLN:HG2   | 2:A:179:HOH:O   | 1.92        | 0.69     |
| 1:B:69:THR:HG23  | 1:B:87:GLY:HA2  | 1.74        | 0.69     |
| 1:A:80:CYS:SG    | 2:A:203:HOH:O   | 2.51        | 0.68     |
| 1:A:124:HIS:HB2  | 2:A:210:HOH:O   | 1.95        | 0.67     |
| 1:A:93:GLU:HA    | 1:A:93:GLU:OE2  | 1.94        | 0.67     |
| 1:A:94:ASP:HB3   | 1:A:95:PRO:CD   | 2.25        | 0.67     |
| 1:B:4:LYS:N      | 2:B:218:HOH:O   | 2.29        | 0.66     |
| 1:A:64:ILE:HD12  | 1:A:91:ALA:HB2  | 1.77        | 0.66     |
| 1:B:81:ILE:HD13  | 1:B:112:TRP:CZ3 | 2.32        | 0.65     |
| 1:A:94:ASP:HB3   | 1:A:95:PRO:HD3  | 1.78        | 0.64     |
| 1:A:80:CYS:HB2   | 2:A:203:HOH:O   | 1.97        | 0.64     |
| 1:B:8:GLU:O      | 1:B:12:SER:OG   | 2.15        | 0.63     |
| 1:A:4:LYS:HB3    | 1:A:5:PRO:HD3   | 1.81        | 0.62     |
| 1:A:102:GLU:OE1  | 1:B:102:GLU:HG2 | 2.02        | 0.59     |
| 1:A:94:ASP:CB    | 1:A:95:PRO:HD3  | 2.32        | 0.59     |
| 1:A:108:ILE:O    | 1:A:109:ASN:HB2 | 2.01        | 0.59     |

*Continued on next page...*

*Continued from previous page...*

| Atom-1           | Atom-2           | Distance(Å) | Clash(Å) |
|------------------|------------------|-------------|----------|
| 1:A:16:ASN:ND2   | 1:A:20:GLN:HE21  | 1.90        | 0.59     |
| 1:B:81:ILE:HD13  | 1:B:112:TRP:CH2  | 2.38        | 0.58     |
| 1:A:106:LYS:HG2  | 1:A:108:ILE:HG13 | 1.84        | 0.58     |
| 1:A:96:ILE:CD1   | 1:A:96:ILE:N     | 2.46        | 0.58     |
| 1:B:90:LYS:HB2   | 1:B:96:ILE:HD11  | 1.87        | 0.57     |
| 1:B:126:PHE:N    | 2:B:228:HOH:O    | 2.31        | 0.57     |
| 1:A:89:LEU:HD12  | 1:A:89:LEU:O     | 2.04        | 0.56     |
| 1:A:8:GLU:O      | 1:A:12:SER:HB2   | 2.06        | 0.56     |
| 1:A:23:ASP:OD1   | 1:A:65:GLN:OE1   | 2.24        | 0.55     |
| 1:B:7:TRP:CD2    | 1:B:75:PRO:HD3   | 2.43        | 0.54     |
| 1:A:84:MET:HG3   | 1:B:102:GLU:OE2  | 2.08        | 0.53     |
| 1:B:80:CYS:O     | 1:B:81:ILE:HD12  | 2.09        | 0.53     |
| 1:A:17:HIS:O     | 1:A:21:LEU:HD13  | 2.10        | 0.52     |
| 1:B:81:ILE:HD12  | 1:B:81:ILE:N     | 2.24        | 0.51     |
| 1:B:12:SER:O     | 1:B:16:ASN:ND2   | 2.43        | 0.51     |
| 1:A:126:PHE:HB3  | 2:B:189:HOH:O    | 2.08        | 0.51     |
| 1:B:89:LEU:O     | 1:B:96:ILE:HD13  | 2.11        | 0.50     |
| 1:B:7:TRP:CE2    | 1:B:75:PRO:HD3   | 2.47        | 0.50     |
| 1:B:125:ASN:ND2  | 2:B:211:HOH:O    | 2.45        | 0.49     |
| 1:A:107:ASN:ND2  | 2:A:153:HOH:O    | 2.44        | 0.49     |
| 1:A:102:GLU:HG3  | 1:B:102:GLU:OE1  | 2.13        | 0.49     |
| 1:B:93:GLU:HB2   | 2:B:226:HOH:O    | 2.12        | 0.49     |
| 1:B:81:ILE:CD1   | 1:B:81:ILE:N     | 2.76        | 0.48     |
| 1:A:64:ILE:CD1   | 1:A:91:ALA:HB2   | 2.42        | 0.48     |
| 1:A:16:ASN:ND2   | 1:A:16:ASN:C     | 2.52        | 0.48     |
| 1:B:61:PHE:CD2   | 1:B:91:ALA:HB1   | 2.48        | 0.48     |
| 1:B:13:SER:HA    | 1:B:16:ASN:HD22  | 1.77        | 0.48     |
| 1:A:26:ARG:HD2   | 1:A:56:LEU:O     | 2.13        | 0.48     |
| 1:B:4:LYS:HE2    | 1:B:5:PRO:HD3    | 1.95        | 0.48     |
| 1:A:6:ILE:HD13   | 1:A:6:ILE:O      | 2.14        | 0.48     |
| 1:A:41:TRP:CD1   | 1:A:42:GLU:HG3   | 2.48        | 0.47     |
| 1:B:40:THR:HA    | 1:B:44:GLN:O     | 2.14        | 0.47     |
| 1:A:77:PRO:HG2   | 1:B:115:THR:HG21 | 1.96        | 0.47     |
| 1:B:40:THR:HG22  | 2:B:162:HOH:O    | 2.14        | 0.47     |
| 1:B:4:LYS:HG3    | 2:B:167:HOH:O    | 2.14        | 0.46     |
| 1:B:25:ASP:HB3   | 1:B:28:GLN:NE2   | 2.31        | 0.46     |
| 1:B:66:HIS:HD2   | 1:B:89:LEU:HD13  | 1.71        | 0.46     |
| 1:A:68:ILE:HD11  | 1:A:85:VAL:HG11  | 1.89        | 0.46     |
| 1:A:104:LEU:CB   | 2:A:204:HOH:O    | 2.44        | 0.46     |
| 1:B:104:LEU:HD23 | 1:B:116:ASN:ND2  | 2.31        | 0.46     |
| 1:A:120:ARG:HG3  | 1:B:84:MET:SD    | 2.56        | 0.46     |
| 1:B:4:LYS:HE2    | 1:B:5:PRO:CD     | 2.46        | 0.45     |

*Continued on next page...*



*Continued from previous page...*

| Atom-1          | Atom-2          | Distance(Å) | Clash(Å) |
|-----------------|-----------------|-------------|----------|
| 1:B:76:THR:OG1  | 1:B:80:CYS:HB2  | 2.17        | 0.45     |
| 1:A:109:ASN:O   | 1:A:110:ASP:HB2 | 2.18        | 0.44     |
| 1:B:26:ARG:NH1  | 1:B:56:LEU:O    | 2.51        | 0.44     |
| 1:A:6:ILE:CD1   | 1:A:10:ILE:HD11 | 2.48        | 0.44     |
| 1:B:4:LYS:HB3   | 1:B:5:PRO:CD    | 2.42        | 0.43     |
| 1:A:25:ASP:OD1  | 1:A:28:GLN:HG2  | 2.18        | 0.43     |
| 1:B:125:ASN:O   | 1:B:126:PHE:CB  | 2.66        | 0.42     |
| 1:A:101:GLN:HG2 | 1:A:103:PHE:CZ  | 2.54        | 0.42     |
| 1:A:68:ILE:HA   | 1:A:68:ILE:HD13 | 1.88        | 0.42     |
| 1:B:39:LEU:HD22 | 1:B:40:THR:N    | 2.33        | 0.42     |
| 1:A:6:ILE:HD11  | 1:A:10:ILE:HD11 | 2.01        | 0.42     |
| 1:A:29:LEU:HD13 | 1:A:56:LEU:HD13 | 2.02        | 0.41     |
| 1:A:94:ASP:OD1  | 1:A:96:ILE:CD1  | 2.68        | 0.41     |
| 1:A:32:ILE:C    | 1:A:32:ILE:HD12 | 2.41        | 0.41     |
| 1:B:67:SER:OG   | 1:B:88:GLN:HB2  | 2.21        | 0.41     |
| 1:A:80:CYS:HB3  | 1:A:105:LEU:O   | 2.20        | 0.41     |
| 1:B:94:ASP:HB3  | 2:B:147:HOH:O   | 2.20        | 0.41     |
| 1:A:68:ILE:HD12 | 1:A:68:ILE:HG23 | 1.85        | 0.41     |
| 1:A:39:LEU:HB3  | 1:A:52:ILE:HG12 | 2.02        | 0.41     |
| 1:B:30:GLY:CA   | 1:B:49:LYS:HE3  | 2.51        | 0.41     |
| 1:A:124:HIS:NE2 | 2:A:155:HOH:O   | 2.37        | 0.41     |
| 1:A:64:ILE:HD12 | 1:A:91:ALA:CB   | 2.49        | 0.41     |
| 1:B:104:LEU:HB3 | 1:B:116:ASN:HB2 | 2.03        | 0.41     |
| 1:B:101:GLN:HA  | 1:B:118:MET:O   | 2.21        | 0.41     |

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               | Distance(Å) | Clash(Å) |
|---------------|----------------------|-------------|----------|
| 2:B:153:HOH:O | 2:B:226:HOH:O[1 554] | 2.12        | 0.08     |

## 5.3 Torsion angles

### 5.3.1 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 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     | 121/127 (95%) | 112 (93%) | 7 (6%)  | 2 (2%)   | 14          | 6   |
| 1   | B     | 121/127 (95%) | 115 (95%) | 6 (5%)  | 0        | 100         | 100 |
| All | All   | 242/254 (95%) | 227 (94%) | 13 (5%) | 2 (1%)   | 27          | 24  |

All (2) Ramachandran outliers are listed below:

| Mol | Chain | Res | Type |
|-----|-------|-----|------|
| 1   | A     | 95  | PRO  |
| 1   | A     | 94  | ASP  |

### 5.3.2 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 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     | 109/111 (98%) | 98 (90%)  | 11 (10%) | 11          | 7  |
| 1   | B     | 109/111 (98%) | 100 (92%) | 9 (8%)   | 16          | 12 |
| All | All   | 218/222 (98%) | 198 (91%) | 20 (9%)  | 13          | 9  |

All (20) residues with a non-rotameric sidechain are listed below:

| Mol | Chain | Res | Type |
|-----|-------|-----|------|
| 1   | A     | 4   | LYS  |
| 1   | A     | 6   | ILE  |
| 1   | A     | 7   | TRP  |
| 1   | A     | 12  | SER  |
| 1   | A     | 16  | ASN  |
| 1   | A     | 54  | GLU  |
| 1   | A     | 88  | GLN  |
| 1   | A     | 95  | PRO  |
| 1   | A     | 96  | ILE  |
| 1   | A     | 107 | ASN  |
| 1   | A     | 125 | ASN  |
| 1   | B     | 6   | ILE  |
| 1   | B     | 12  | SER  |
| 1   | B     | 21  | LEU  |
| 1   | B     | 39  | LEU  |

*Continued on next page...*

*Continued from previous page...*

| Mol | Chain | Res | Type |
|-----|-------|-----|------|
| 1   | B     | 44  | GLN  |
| 1   | B     | 54  | GLU  |
| 1   | B     | 96  | ILE  |
| 1   | B     | 110 | ASP  |
| 1   | B     | 125 | ASN  |

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (9) such sidechains are listed below:

| Mol | Chain | Res | Type |
|-----|-------|-----|------|
| 1   | A     | 20  | GLN  |
| 1   | A     | 47  | GLN  |
| 1   | A     | 73  | HIS  |
| 1   | A     | 107 | ASN  |
| 1   | A     | 116 | ASN  |
| 1   | A     | 125 | ASN  |
| 1   | B     | 16  | ASN  |
| 1   | B     | 109 | ASN  |
| 1   | B     | 116 | ASN  |

### 5.3.3 RNA ⓘ

There are no RNA chains 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 ⓘ

EDS was not executed - this section will therefore be empty.

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

EDS was not executed - this section will therefore be empty.

### 6.3 Carbohydrates ⓘ

EDS was not executed - this section will therefore be empty.

### 6.4 Ligands ⓘ

EDS was not executed - this section will therefore be empty.

### 6.5 Other polymers ⓘ

EDS was not executed - this section will therefore be empty.