

wwPDB NMR Structure Validation Summary Report (i)

Aug 8, 2023 – 03:12 pm BST

PDB ID : 8OS0 BMRB ID : 34808

Title : Solution NMR structure of Notch3 WT TMD Authors : Guschtschin-Schmidt, N.; Muhle-Goll, C.

Deposited on : 2023-04-17

This is a wwPDB NMR Structure Validation Summary Report for a publicly released PDB entry.

We welcome your comments at validation@mail.wwpdb.org
A user guide is available at
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 (1)) 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)

 $\begin{array}{ccc} wwPDB\text{-}ShiftChecker &:& v1.2\\ BMRB \ Restraints \ Analysis &:& v1.2 \end{array}$

Ideal geometry (proteins) : Engh & Huber (2001) Ideal geometry (DNA, RNA) : Parkinson et al. (1996)

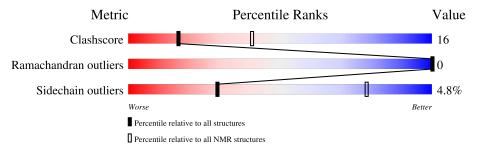
Validation Pipeline (wwPDB-VP) : 2.35

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 | Whole archive | NMR archive |
|-----------------------|----------------------|----------------------|
| Metric | $(\# 	ext{Entries})$ | $(\# 	ext{Entries})$ |
| 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 | 30 | 30% | 17% | 53% | |



2 Ensemble composition and analysis (i)

This entry contains 40 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 | Well-defined core Residue range (total) Backbone RMSD (Å) Medoid model | | | | |
| 1 | A:1648-A:1661 (14) | 0.34 | 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 5 clusters. No single-model clusters were found.

| Cluster number | Models |
|----------------|--|
| 1 | 1, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, |
| <u>+</u> | 21, 22, 23, 25, 27, 29, 30, 31, 33, 35, 39 |
| 2 | 2, 26, 28, 32, 34, 36, 40 |
| 3 | 5, 38 |
| 4 | 11, 20 |
| 5 | 24, 37 |



3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 510 atoms, of which 288 are hydrogens and 0 are deuteriums.

• Molecule 1 is a protein called Notch 3 extracellular truncation.

| Mol | Chain | Residues | Atoms | | | | | Trace | | |
|-----|-------|----------|-------|-----|-----|-----|----|-------|---|---|
| 1 | Λ | 20 | Total | С | Н | N | О | S | 0 | |
| 1 | A | 1 A | 30 | 510 | 154 | 288 | 36 | 31 | 1 | U |

There are 6 discrepancies between the modelled and reference sequences:

| Chain | Residue | Modelled | Actual | Comment | Reference |
|-------|---------|----------|--------|----------------|------------|
| A | 1639 | LYS | - | expression tag | UNP Q9UM47 |
| A | 1640 | LYS | - | expression tag | UNP Q9UM47 |
| A | 1641 | LYS | - | expression tag | UNP Q9UM47 |
| A | 1666 | LYS | - | expression tag | UNP Q9UM47 |
| A | 1667 | LYS | - | expression tag | UNP Q9UM47 |
| A | 1668 | LYS | - | expression tag | UNP Q9UM47 |



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.

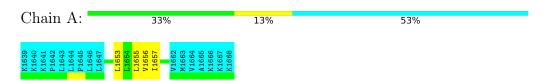
• Molecule 1: Notch 3 extracellular truncation



4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 8. Colouring as in section 4.1 above.

• Molecule 1: Notch 3 extracellular truncation





Refinement protocol and experimental data overview (i) 5



The models were refined using the following method: simulated annealing.

Of the 400 calculated structures, 40 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 |
|---------------|-----------------------|---------|
| CNS | refinement | 1.2.1 |
| ARIA | structure calculation | 2.3.2 |

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 | 433 |
| Number of shifts mapped to atoms | 433 |
| 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% |



6 Model quality (i)

6.1 Standard geometry (i)

There are no covalent bond-length or bond-angle outliers.

There are no bond-length outliers.

There are no bond-angle outliers.

There are no chirality outliers.

There are no planarity outliers.

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 |
|-----|-------|-------|----------|----------|---------|
| 1 | A | 94 | 118 | 118 | 3±1 |
| All | All | 3760 | 4720 | 4720 | 133 |

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 16.

5 of 16 unique clashes are listed below, sorted by their clash magnitude.

| Atom-1 | Atom-2 | Clash(Å) | Distance(Å) | Models | | |
|-------------------|-------------------|----------|-------------|--------|-------|--|
| Atom-1 | Atom-2 | Clash(A) | Distance(A) | Worst | Total | |
| 1:A:1655:LEU:C | 1:A:1655:LEU:HD13 | 0.56 | 2.21 | 35 | 2 | |
| 1:A:1656:VAL:HG13 | 1:A:1657:ILE:N | 0.55 | 2.17 | 6 | 31 | |
| 1:A:1652:VAL:O | 1:A:1656:VAL:HG23 | 0.51 | 2.06 | 11 | 1 | |
| 1:A:1654:LEU:O | 1:A:1658:LEU:HB3 | 0.48 | 2.08 | 7 | 14 | |
| 1:A:1653:LEU:HA | 1:A:1656:VAL:HG12 | 0.47 | 1.87 | 19 | 29 | |

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 | Outliers Percen | |
|-----|-------|----------------|--------------|------------|------------|-----------------|-----|
| 1 | A | 14/30 (47%) | 13±1 (92±6%) | 1±1 (8±6%) | 0±0 (0±0%) | 100 | 100 |
| All | All | 560/1200 (47%) | 514 (92%) | 46 (8%) | 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 | Perce | ntiles |
|-----|-------|----------------|--------------|------------|-------|--------|
| 1 | A | 10/25 (40%) | 10±0 (95±5%) | 0±0 (5±5%) | 29 | 78 |
| All | All | 400/1000 (40%) | 381 (95%) | 19 (5%) | 29 | 78 |

All 5 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 | 1658 | LEU | 5 |
| 1 | A | 1655 | LEU | 5 |
| 1 | A | 1656 | VAL | 4 |
| 1 | A | 1654 | LEU | 3 |
| 1 | A | 1660 | LEU | 2 |

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 82% for the well-defined parts and 80% for the entire structure.

7.1 Chemical shift list 1

File name: working cs.cif

Chemical shift list name: Notch3.str

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 | 433 |
|---|-----|
| Number of shifts mapped to atoms | 433 |
| 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 |

The following errors were found when reading this chemical shift list.

• Chemical shift has been reported more than once. First 5 (of 0) occurrences are reported below.

| T:a4 ID | Clasia. | Dag | Т | Atom | | Shift Data | | |
|---------|---------|------|------|------|-------|-------------|-----------|--|
| List ID | Chain | Res | Type | Atom | Value | Uncertainty | Ambiguity | |
| 1 | A | 1643 | LEU | HD12 | 0.967 | | • | |
| 1 | A | 1643 | LEU | HD13 | 0.967 | | | |
| 1 | A | 1643 | LEU | HD22 | 0.906 | • | • | |
| 1 | A | 1643 | LEU | HD23 | 0.906 | | | |
| 1 | A | 1644 | LEU | HD12 | 0.982 | • | • | |
| 1 | A | 1644 | LEU | HD13 | 0.982 | | | |
| 1 | A | 1644 | LEU | HD22 | 0.919 | • | • | |
| 1 | A | 1644 | LEU | HD23 | 0.919 | • | | |
| 1 | A | 1646 | LEU | HD12 | 0.968 | • | • | |
| 1 | A | 1646 | LEU | HD13 | 0.968 | | | |
| 1 | A | 1646 | LEU | HD22 | 0.893 | • | • | |
| 1 | A | 1646 | LEU | HD23 | 0.893 | | | |
| 1 | A | 1647 | LEU | HD12 | 0.919 | • | • | |
| 1 | A | 1647 | LEU | HD13 | 0.919 | | • | |

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| List ID Chain Res Type Atom 1 A 1647 LEU HD22 1 A 1647 LEU HD23 1 A 1648 VAL HG12 1 A 1648 VAL HG13 1 A 1648 VAL HG22 1 A 1648 VAL HG23 1 A 1649 ALA HB2 1 A 1651 ALA HB3 1 A 1651 ALA HB3 1 A 1652 VAL HG12 1 A 1652 VAL HG13 | Value 0.877 0.877 0.940 0.940 1.007 1.507 1.507 1.559 1.559 | Uncertainty | Ambiguity |
|--|--|-------------|-----------|
| 1 A 1647 LEU HD23 1 A 1648 VAL HG12 1 A 1648 VAL HG13 1 A 1648 VAL HG22 1 A 1648 VAL HG23 1 A 1649 ALA HB2 1 A 1659 ALA HB3 1 A 1651 ALA HB3 1 A 1651 ALA HB3 1 A 1652 VAL HG12 | 0.877 0.940 0.940 1.007 1.007 1.507 1.507 1.559 | | |
| 1 A 1648 VAL HG12 1 A 1648 VAL HG13 1 A 1648 VAL HG22 1 A 1648 VAL HG23 1 A 1649 ALA HB2 1 A 1649 ALA HB3 1 A 1651 ALA HB2 1 A 1651 ALA HB3 1 A 1652 VAL HG12 | 0.940 0.940 1.007 1.007 1.507 1.507 1.559 | | |
| 1 A 1648 VAL HG13 1 A 1648 VAL HG22 1 A 1648 VAL HG23 1 A 1649 ALA HB2 1 A 1649 ALA HB3 1 A 1651 ALA HB3 1 A 1651 ALA HB3 1 A 1652 VAL HG12 | 0.940 1.007 1.007 1.507 1.507 1.559 | | |
| 1 A 1648 VAL HG22 1 A 1648 VAL HG23 1 A 1649 ALA HB2 1 A 1649 ALA HB3 1 A 1651 ALA HB2 1 A 1651 ALA HB3 1 A 1652 VAL HG12 | 1.007 1.007 1.507 1.507 1.559 | | |
| 1 A 1648 VAL HG23 1 A 1649 ALA HB2 1 A 1649 ALA HB3 1 A 1651 ALA HB2 1 A 1651 ALA HB3 1 A 1652 VAL HG12 | 1.007 1.507 1.507 1.559 | | |
| 1 A 1649 ALA HB2 1 A 1649 ALA HB3 1 A 1651 ALA HB2 1 A 1651 ALA HB3 1 A 1652 VAL HG12 | 1.507 1.507 1.559 | | |
| 1 A 1649 ALA HB3 1 A 1651 ALA HB2 1 A 1651 ALA HB3 1 A 1652 VAL HG12 | 1.507 1.559 | | |
| 1 A 1651 ALA HB2 1 A 1651 ALA HB3 1 A 1652 VAL HG12 | 1.559 | • | |
| 1 A 1651 ALA HB3 1 A 1652 VAL HG12 | | | |
| 1 A 1652 VAL HG12 | 1.559 | • | |
| | | • | |
| 1 A 1659 VAT HOTO | 1.056 | • | |
| 1 A 1652 VAL HG13 | 1.056 | • | |
| 1 A 1652 VAL HG22 | 0.944 | • | |
| 1 A 1652 VAL HG23 | 0.944 | | |
| 1 A 1653 LEU HD12 | 0.944 | | |
| 1 A 1653 LEU HD13 | 0.944 | | |
| 1 A 1653 LEU HD22 | 0.902 | • | |
| 1 A 1653 LEU HD23 | 0.902 | | |
| 1 A 1654 LEU HD12 | 0.936 | • | |
| 1 A 1654 LEU HD13 | 0.936 | • | |
| 1 A 1654 LEU HD22 | 0.898 | • | |
| 1 A 1654 LEU HD23 | 0.898 | • | |
| 1 A 1655 LEU HD12 | 0.934 | | |
| 1 A 1655 LEU HD13 | 0.934 | • | |
| 1 A 1655 LEU HD22 | 0.899 | • | |
| 1 A 1655 LEU HD23 | 0.899 | • | |
| 1 A 1656 VAL HG12 | 1.077 | • | |
| 1 A 1656 VAL HG13 | 1.077 | • | |
| 1 A 1656 VAL HG22 | 0.937 | • | |
| 1 A 1656 VAL HG23 | 0.937 | • | |
| 1 A 1657 ILE HD12 | 0.845 | • | |
| 1 A 1657 ILE HD13 | 0.845 | • | |
| 1 A 1657 ILE HG22 | 0.940 | • | |
| 1 A 1657 ILE HG23 | 0.940 | | |
| 1 A 1658 LEU HD12 | 0.888 | | |
| 1 A 1658 LEU HD13 | 0.888 | | |
| 1 A 1659 VAL HG12 | 1.080 | | |
| 1 A 1659 VAL HG13 | 1.080 | | |
| 1 A 1659 VAL HG22 | 0.965 | <u>.</u> | |
| 1 A 1659 VAL HG23 | 0.965 | <u> </u> | |
| 1 A 1660 LEU HD12 | 0.869 | • | |

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| List ID | Chain | Dag | Type Atom | | | Shift Dat | a |
|---------|-------|------|-----------|------|-------|-------------|-----------|
| LIST ID | Chain | Res | Type | Atom | Value | Uncertainty | Ambiguity |
| 1 | A | 1660 | LEU | HD13 | 0.869 | | |
| 1 | A | 1662 | VAL | HG12 | 1.096 | • | |
| 1 | A | 1662 | VAL | HG13 | 1.096 | • | |
| 1 | A | 1662 | VAL | HG22 | 0.968 | • | |
| 1 | A | 1662 | VAL | HG23 | 0.968 | • | |
| 1 | A | 1664 | VAL | HG12 | 1.082 | • | |
| 1 | A | 1664 | VAL | HG13 | 1.082 | • | |
| 1 | A | 1664 | VAL | HG22 | 0.955 | • | • |
| 1 | A | 1664 | VAL | HG23 | 0.955 | • | |
| 1 | A | 1665 | ALA | HB2 | 1.549 | • | |
| 1 | A | 1665 | ALA | HB3 | 1.549 | | |

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

| Nucleus | # values | Correction \pm precision, ppm | Suggested action |
|----------------------------|----------|-----------------------------------|-------------------------------------|
| $^{13}\mathrm{C}_{\alpha}$ | 30 | 0.12 ± 0.09 | None needed ($< 0.5 \text{ ppm}$) |
| $^{13}C_{\beta}$ | 28 | 0.81 ± 0.12 | Should be checked |
| ¹³ C′ | 0 | | None (insufficient data) |
| ^{15}N | 28 | 0.60 ± 0.57 | None needed (imprecise) |

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 82%, i.e. 163 atoms were assigned a chemical shift out of a possible 198. 0 out of 9 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

| | Total | $^{1}{ m H}$ | $^{13}\mathbf{C}$ | $^{15}{ m N}$ |
|-----------|---------------|---------------|-------------------|---------------|
| Backbone | 57/72 (79%) | 29/30 (97%) | 14/28~(50%) | 14/14 (100%) |
| Sidechain | 106/126 (84%) | 75/88 (85%) | 31/38 (82%) | 0/0 (%) |
| Overall | 163/198 (82%) | 104/118 (88%) | 45/66 (68%) | 14/14 (100%) |

7.1.4 Statistically unusual chemical shifts (i)

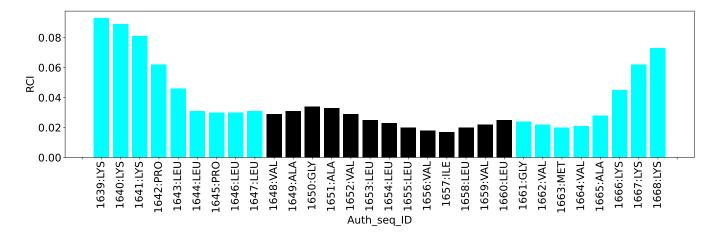
There are no statistically unusual chemical shifts.



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 (i)

No restraints data found



9 Distance violation analysis (i)

No distance restraints data found



10 Dihedral-angle violation analysis (i)

No dihedral-angle restraints found

