Full wwPDB NMR Structure Validation Report (i)

May 29, 2020 – 12:37 am BST

PDB ID	:	2OI3
Title	:	NMR Structure Analysis of the Hematopoetic Cell Kinase SH3 Domain complexed with an artificial high affinity ligand (PD1)
Authors	:	Schmidt, H.; Stoldt, M.; Hoffmann, S.; Tran, T.; Willbold, D.
Deposited on	:	2007-01-10

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

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
https://www.wwpdb.org/validation/2017/NMRValidationReportHelp
with specific help available everywhere you see the (i) symbol.

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

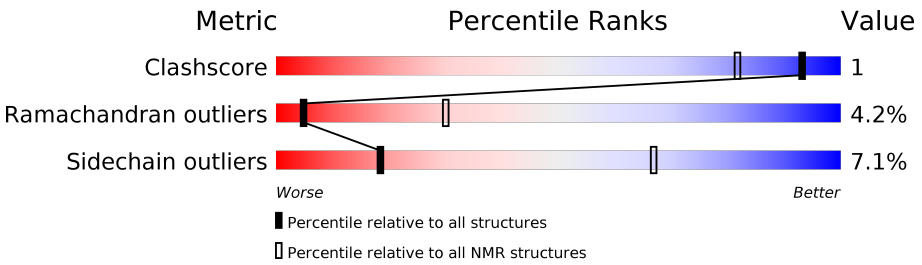
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	2.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.11

1 Overall quality at a glance (i)

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

The overall completeness of chemical shifts assignment is 79%.

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	86	87% 10% •
2	B	14	86% 7% 7%

2 Ensemble composition and analysis (i)

This entry contains 1 models. Identification of well-defined residues and clustering analysis are not possible.

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 1507 atoms, of which 738 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Tyrosine-protein kinase HCK.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	86	1303	417	633	115	137	1	0

There are 5 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1	GLY	-	cloning artifact	UNP P08631
A	2	PRO	-	cloning artifact	UNP P08631
A	3	LEU	-	cloning artifact	UNP P08631
A	4	GLY	-	cloning artifact	UNP P08631
A	5	SER	-	cloning artifact	UNP P08631

- Molecule 2 is a protein called artificial peptide PD1.

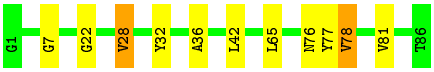
Mol	Chain	Residues	Atoms					Trace
			Total	C	H	N	O	
2	B	14	204	67	105	16	16	1

4 Residue-property plots (i)

These plots are provided for all protein, RNA and DNA chains in the entry. The first graphic is the same as shown in the summary in section 1 of this report. The second graphic shows the sequence where residues are colour-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. Stretches of 2 or more consecutive residues without any outliers are shown as green connectors. Residues which are classified as ill-defined in the NMR ensemble, are shown in cyan with an underline colour-coded according to the previous scheme. Residues which were present in the experimental sample, but not modelled in the final structure are shown in grey.

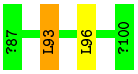
- Molecule 1: Tyrosine-protein kinase HCK

Chain A:



- Molecule 2: artificial peptide PD1

Chain B:



5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *Simulated annealing with generalized born solvent model and NMR distance constraints.*

Of the 20 calculated structures, 1 were deposited, based on the following criterion: *Minimized average structure.*

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

Software name	Classification	Version
RADAR	refinement	0.9b
Amber	refinement	8.0
CYANA	structure solution	1.1

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

Chemical shift file(s)	input_cs.cif
Number of chemical shift lists	1
Total number of shifts	1033
Number of shifts mapped to atoms	1033
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	79%

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

COVALENT-GEOMETRY INFOmissingINFO

5.1 Too-close contacts (i)

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in each chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes averaged over the ensemble.

Mol	Chain	Non-H	H(model)	H(added)	Clashes
1	A	670	633	632	2
2	B	99	105	103	2
All	All	769	738	735	2

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including

hydrogen atoms). The all-atom clashscore for this structure is 1.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)
1:A:76:ASN:HB3	2:B:93:LEU:HD13	0.40	1.92
1:A:32:TYR:CE2	2:B:96:LEU:HD22	0.40	2.51

5.2 Torsion angles (i)

5.2.1 Protein backbone (i)

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all NMR entries. The Analysed column shows the number of residues for which the backbone conformation was analysed and the total number of residues.

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles
1	A	84/86 (98%)	69 (82%)	12 (14%)	3 (4%)	6 34
2	B	12/14 (86%)	10 (83%)	1 (8%)	1 (8%)	2 13
All	All	96/100 (96%)	79 (82%)	13 (14%)	4 (4%)	5 30

All 4 Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type
1	A	7	GLY
2	B	93	LEU
1	A	22	GLY
1	A	36	ALA

5.2.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all NMR entries. The Analysed column shows the number of residues for which the sidechain conformation was analysed and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	A	72/72 (100%)	66 (92%)	6 (8%)	15 62
2	B	12/12 (100%)	12 (100%)	0 (0%)	100 100
All	All	84/84 (100%)	78 (93%)	6 (7%)	18 67

All 6 residues with a non-rotameric sidechain are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type
1	A	81	VAL
1	A	65	LEU
1	A	78	VAL
1	A	28	VAL
1	A	42	LEU
1	A	77	TYR

5.2.3 RNA (i)

There are no RNA molecules in this entry.

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

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

5.4 Carbohydrates (i)

There are no carbohydrates in this entry.

5.5 Ligand geometry (i)

There are no ligands in this entry.

5.6 Other polymers (i)

There are no such molecules in this entry.

5.7 Polymer linkage issues (i)

There are no chain breaks in this entry.

6 Chemical shift validation (i)

The completeness of assignment taking into account all chemical shift lists is 79% for the well-defined parts and 79% for the entire structure.

6.1 Chemical shift list 1

File name: input_cs.cif

Chemical shift list name: *assigned_chem_shift_list_1*

6.1.1 Bookkeeping (i)

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

Total number of shifts	1033
Number of shifts mapped to atoms	1033
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	6

6.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	86	0.00 ± 0.23	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	78	-0.03 ± 0.20	None needed (< 0.5 ppm)
$^{13}C'$	79	0.23 ± 0.16	None needed (< 0.5 ppm)
^{15}N	79	0.35 ± 0.28	None needed (< 0.5 ppm)

6.1.3 Completeness of resonance assignments (i)

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 79%, i.e. 930 atoms were assigned a chemical shift out of a possible 1173. 12 out of 15 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	409/472 (87%)	165/187 (88%)	165/196 (84%)	79/89 (89%)
Sidechain	454/599 (76%)	281/355 (79%)	163/222 (73%)	10/22 (45%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	67/102 (66%)	39/53 (74%)	26/42 (62%)	2/7 (29%)
Overall	930/1173 (79%)	485/595 (82%)	354/460 (77%)	91/118 (77%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 79%, i.e. 930 atoms were assigned a chemical shift out of a possible 1173. 12 out of 15 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	409/472 (87%)	165/187 (88%)	165/196 (84%)	79/89 (89%)
Sidechain	454/599 (76%)	281/355 (79%)	163/222 (73%)	10/22 (45%)
Aromatic	67/102 (66%)	39/53 (74%)	26/42 (62%)	2/7 (29%)
Overall	930/1173 (79%)	485/595 (82%)	354/460 (77%)	91/118 (77%)

6.1.4 Statistically unusual chemical shifts (i)

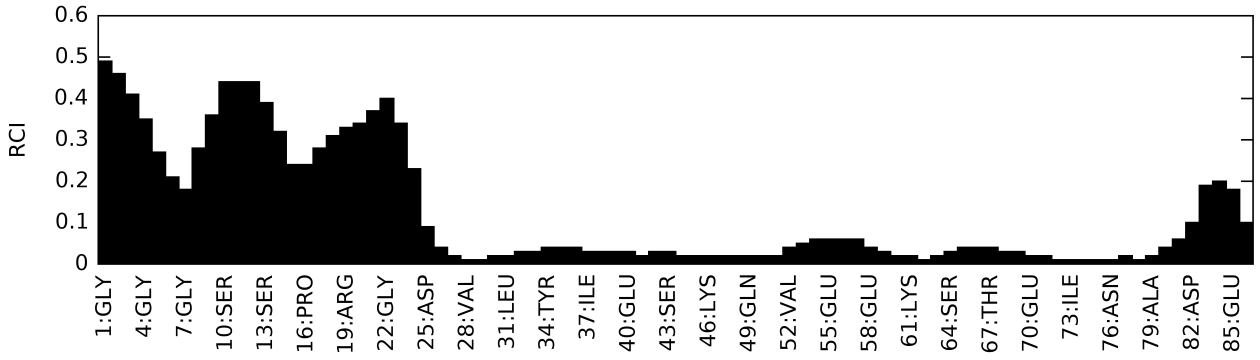
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	75	SER	HB2	1.59	5.18 – 2.58	-8.8
1	A	68	ARG	NE	72.00	92.63 – 76.73	-8.0
1	A	75	SER	HB3	2.15	5.25 – 2.45	-6.1
1	A	34	TYR	HB2	0.71	4.76 – 1.06	-5.9
1	A	74	PRO	HG2	0.21	3.48 – 0.38	-5.5
1	A	74	PRO	HB2	0.17	3.82 – 0.32	-5.4

6.1.5 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.

Random coil index (RCI) for chain A:


RCI
0.6
0.5
0.4
0.3
0.2
0.1
0
1:GLY
4:GLY
7:GLY
10:SER
13:SER
16:PRO
19:ARG
22:GLY
25:ASP
28:VAL
31:LEU
34:TYR
37:ILE
40:GLU
43:SER
46:LYS
49:GLN
52:VAL
55:GLU
58:GLU
61:LYS
64:SER
67:THR
70:GLU
73:ILE
76:ASN
79:ALA
82:ASP
85:GLU