wwPDB NMR Structure Validation Summary Report

Jun 4, 2023 – 09:11 PM EDT

PDB ID	:	2LP5
BMRB ID	:	17149
Title	:	Native Structure of the Fyn SH3 A39V/N53P/V55L
Authors	:	Neudecker, P.; Robustelli, P.; Cavalli, A.; Vendruscolo, M.; Kay, L.E.
Deposited on	:	2012-02-06

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 (i)) were used in the production of this report:

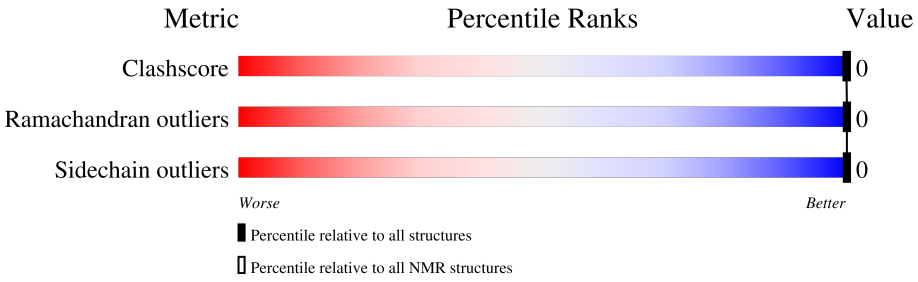
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
BMRB Restraints Analysis	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal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

1 Overall quality at a glance (i)

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

The overall completeness of chemical shifts assignment is 75%.

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	66	85% 5% 11%

2 Ensemble composition and analysis

This entry contains 10 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: *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:58 (56)	0.31	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 4 single-model clusters were found.

Cluster number	Models
1	4, 5, 6
2	1, 8, 10
Single-model clusters	2; 3; 7; 9

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 917 atoms, of which 437 are hydrogens and 0 are deuteriums.

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

Mol	Chain	Residues	Atoms					Trace
			Total	C	H	N	O	
1	A	59	917	307	437	73	100	0

There are 11 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-5	GLY	-	expression tag	UNP Q05876
A	-4	ALA	-	expression tag	UNP Q05876
A	-3	MET	-	expression tag	UNP Q05876
A	-2	VAL	-	expression tag	UNP Q05876
A	-1	GLN	-	expression tag	UNP Q05876
A	0	ILE	-	expression tag	UNP Q05876
A	1	SER	-	expression tag	UNP Q05876
A	39	VAL	ALA	engineered mutation	UNP Q05876
A	53	PRO	ASN	engineered mutation	UNP Q05876
A	55	LEU	VAL	engineered mutation	UNP Q05876
A	60	ARG	-	expression tag	UNP Q05876

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: Tyrosine-protein kinase Fyn

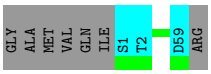
Chain A:

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: Tyrosine-protein kinase Fyn

Chain A:



5 Refinement protocol and experimental data overview

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

Of the 1000 calculated structures, 10 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
ALMOST	structure solution	2.0
ALMOST	refinement	2.0

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	4
Total number of shifts	2269
Number of shifts mapped to atoms	2041
Number of unparsed shifts	0
Number of shifts with mapping errors	228
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	75%

6 Model quality

6.1 Standard geometry

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

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	4590	4210	4210	-

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	56/66 (85%)	56±0 (100±0%)	0±0 (0±0%)	0±0 (0±0%)	100	100
All	All	560/660 (85%)	560 (100%)	0 (0%)	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	49/57 (86%)	49±0 (100±0%)	0±0 (0±0%)	100	100
All	All	490/570 (86%)	490 (100%)	0 (0%)	100	100

There are no protein residues with a non-rotameric sidechain to report.

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

The completeness of assignment taking into account all chemical shift lists is 75% for the well-defined parts and 75% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *assignments_fynsh3a39vn53pv55l*

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	664
Number of shifts mapped to atoms	588
Number of unparsed shifts	0
Number of shifts with mapping errors	76
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	4

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

- No matching atom found in the structure. First 5 (of 76) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	-4	ALA	HA	4.32	0.01	1
1	A	-4	ALA	HB1	1.35	0.01	1
1	A	-4	ALA	HB2	1.35	0.01	1
1	A	-4	ALA	HB3	1.35	0.01	1
1	A	-4	ALA	C	177.62	0.10	1
1	A	-4	ALA	CA	52.26	0.10	1
1	A	-4	ALA	CB	19.47	0.10	1
1	A	-3	MET	H	8.49	0.01	1
1	A	-3	MET	HA	4.45	0.01	1
1	A	-3	MET	HB2	2.0	0.01	2
1	A	-3	MET	HB3	2.0	0.01	2
1	A	-3	MET	HE1	2.05	0.01	1
1	A	-3	MET	HE2	2.05	0.01	1
1	A	-3	MET	HE3	2.05	0.01	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	-3	MET	C	176.08	0.10	1
1	A	-3	MET	CA	55.36	0.10	1
1	A	-3	MET	CE	16.99	0.10	1
1	A	-3	MET	N	120.72	0.10	1
1	A	-2	VAL	H	8.19	0.01	1
1	A	-2	VAL	HA	4.07	0.01	1
1	A	-2	VAL	HB	1.99	0.01	1
1	A	-2	VAL	HG11	0.86	0.01	1
1	A	-2	VAL	HG12	0.86	0.01	1
1	A	-2	VAL	HG13	0.86	0.01	1
1	A	-2	VAL	HG21	0.87	0.01	1
1	A	-2	VAL	HG22	0.87	0.01	1
1	A	-2	VAL	HG23	0.87	0.01	1
1	A	-2	VAL	C	175.75	0.10	1
1	A	-2	VAL	CA	62.12	0.10	1
1	A	-2	VAL	CG1	21.18	0.10	1
1	A	-2	VAL	CG2	20.62	0.10	1
1	A	-2	VAL	N	122.74	0.10	1
1	A	-1	GLN	H	8.51	0.01	1
1	A	-1	GLN	HA	4.34	0.01	1
1	A	-1	GLN	HB2	1.96	0.01	2
1	A	-1	GLN	HB3	1.96	0.01	2
1	A	-1	GLN	HE21	7.51	0.01	2
1	A	-1	GLN	HE22	6.83	0.01	2
1	A	-1	GLN	HG2	2.3	0.01	2
1	A	-1	GLN	HG3	2.3	0.01	2
1	A	-1	GLN	C	175.88	0.10	1
1	A	-1	GLN	CA	55.5	0.10	1
1	A	-1	GLN	CD	180.37	0.10	1
1	A	-1	GLN	CG	33.4	0.10	1
1	A	-1	GLN	N	124.92	0.10	1
1	A	-1	GLN	NE2	112.61	0.10	1
1	A	0	ILE	H	8.33	0.01	1
1	A	0	ILE	HA	4.18	0.01	1
1	A	0	ILE	HB	1.85	0.01	1
1	A	0	ILE	HD11	0.75	0.01	1
1	A	0	ILE	HD12	0.75	0.01	1
1	A	0	ILE	HD13	0.75	0.01	1
1	A	0	ILE	HG12	1.42	0.01	2
1	A	0	ILE	HG13	1.16	0.01	2
1	A	0	ILE	HG21	0.94	0.01	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	0	ILE	HG22	0.94	0.01	1
1	A	0	ILE	HG23	0.94	0.01	1
1	A	0	ILE	C	176.39	0.10	1
1	A	0	ILE	CA	61.26	0.10	1
1	A	0	ILE	CD1	12.95	0.10	1
1	A	0	ILE	CG2	17.64	0.10	1
1	A	0	ILE	N	123.17	0.10	1
1	A	60	ARG	H	7.61	0.01	1
1	A	60	ARG	HA	4.11	0.01	1
1	A	60	ARG	HB2	1.71	0.01	2
1	A	60	ARG	HB3	1.55	0.01	2
1	A	60	ARG	HD2	3.13	0.01	1
1	A	60	ARG	HD3	3.13	0.01	1
1	A	60	ARG	HE	7.15	0.01	1
1	A	60	ARG	HG2	1.43	0.01	1
1	A	60	ARG	HG3	1.43	0.01	1
1	A	60	ARG	CA	57.03	0.10	1
1	A	60	ARG	CD	43.19	0.10	1
1	A	60	ARG	CZ	159.53	0.10	1
1	A	60	ARG	N	124.96	0.10	1
1	A	60	ARG	NE	84.66	0.10	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
$^{13}C_{\alpha}$	65	0.17 ± 0.18	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	11	—	None (insufficient data)
$^{13}C'$	61	0.02 ± 0.17	None needed (< 0.5 ppm)
^{15}N	61	0.52 ± 0.56	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 75%, i.e. 561 atoms were assigned a chemical shift out of a possible 751. 0 out of 8 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	275/278 (99%)	113/113 (100%)	109/112 (97%)	53/53 (100%)

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	Total	^{1}H	^{13}C	^{15}N
Sidechain	258/376 (69%)	214/243 (88%)	40/123 (33%)	4/10 (40%)
Aromatic	28/97 (29%)	24/47 (51%)	2/47 (4%)	2/3 (67%)
Overall	561/751 (75%)	351/403 (87%)	151/282 (54%)	59/66 (89%)

7.1.4 Statistically unusual chemical shifts (i)

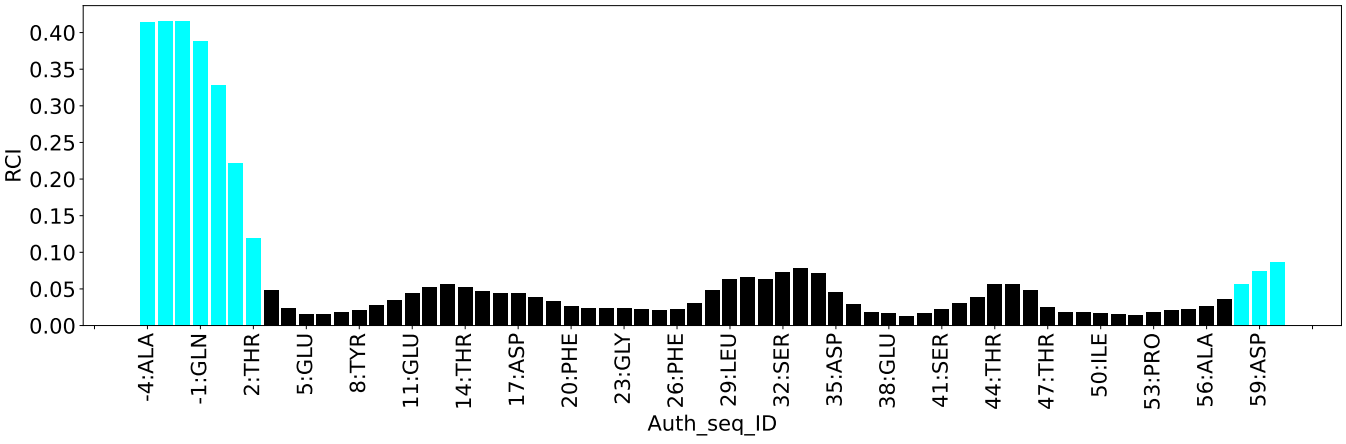
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	52	SER	HB3	1.59	2.49 – 5.20	-8.3
1	A	52	SER	HB2	1.97	2.61 – 5.13	-7.5
1	A	31	SER	HB3	2.19	2.49 – 5.20	-6.1
1	A	10	TYR	HB3	0.56	0.93 – 4.76	-6.0

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:



7.2 Chemical shift list 2

File name: working_cs.cif

Chemical shift list name: *assignments_fynsh3a39vn53pv55l_1*

7.2.1 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	664
Number of shifts mapped to atoms	588
Number of unparsed shifts	0
Number of shifts with mapping errors	76
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	4

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

- No matching atom found in the structure. First 5 (of 76) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
2	A	-4	ALA	HA	4.32	0.01	1
2	A	-4	ALA	HB1	1.35	0.01	1
2	A	-4	ALA	HB2	1.35	0.01	1
2	A	-4	ALA	HB3	1.35	0.01	1
2	A	-4	ALA	C	177.62	0.10	1
2	A	-4	ALA	CA	52.26	0.10	1
2	A	-4	ALA	CB	19.47	0.10	1
2	A	-3	MET	H	8.49	0.01	1
2	A	-3	MET	HA	4.45	0.01	1
2	A	-3	MET	HB2	2.0	0.01	2
2	A	-3	MET	HB3	2.0	0.01	2
2	A	-3	MET	HE1	2.05	0.01	1
2	A	-3	MET	HE2	2.05	0.01	1
2	A	-3	MET	HE3	2.05	0.01	1
2	A	-3	MET	C	176.08	0.10	1
2	A	-3	MET	CA	55.36	0.10	1
2	A	-3	MET	CE	16.99	0.10	1
2	A	-3	MET	N	120.72	0.10	1
2	A	-2	VAL	H	8.19	0.01	1
2	A	-2	VAL	HA	4.07	0.01	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
2	A	-2	VAL	HB	1.99	0.01	1
2	A	-2	VAL	HG11	0.86	0.01	1
2	A	-2	VAL	HG12	0.86	0.01	1
2	A	-2	VAL	HG13	0.86	0.01	1
2	A	-2	VAL	HG21	0.87	0.01	1
2	A	-2	VAL	HG22	0.87	0.01	1
2	A	-2	VAL	HG23	0.87	0.01	1
2	A	-2	VAL	C	175.75	0.10	1
2	A	-2	VAL	CA	62.12	0.10	1
2	A	-2	VAL	CG1	21.18	0.10	1
2	A	-2	VAL	CG2	20.62	0.10	1
2	A	-2	VAL	N	122.74	0.10	1
2	A	-1	GLN	H	8.51	0.01	1
2	A	-1	GLN	HA	4.34	0.01	1
2	A	-1	GLN	HB2	1.96	0.01	2
2	A	-1	GLN	HB3	1.96	0.01	2
2	A	-1	GLN	HE21	7.51	0.01	2
2	A	-1	GLN	HE22	6.83	0.01	2
2	A	-1	GLN	HG2	2.3	0.01	2
2	A	-1	GLN	HG3	2.3	0.01	2
2	A	-1	GLN	C	175.88	0.10	1
2	A	-1	GLN	CA	55.5	0.10	1
2	A	-1	GLN	CD	180.37	0.10	1
2	A	-1	GLN	CG	33.4	0.10	1
2	A	-1	GLN	N	124.92	0.10	1
2	A	-1	GLN	NE2	112.61	0.10	1
2	A	0	ILE	H	8.33	0.01	1
2	A	0	ILE	HA	4.18	0.01	1
2	A	0	ILE	HB	1.85	0.01	1
2	A	0	ILE	HD11	0.75	0.01	1
2	A	0	ILE	HD12	0.75	0.01	1
2	A	0	ILE	HD13	0.75	0.01	1
2	A	0	ILE	HG12	1.42	0.01	2
2	A	0	ILE	HG13	1.16	0.01	2
2	A	0	ILE	HG21	0.94	0.01	1
2	A	0	ILE	HG22	0.94	0.01	1
2	A	0	ILE	HG23	0.94	0.01	1
2	A	0	ILE	C	176.39	0.10	1
2	A	0	ILE	CA	61.26	0.10	1
2	A	0	ILE	CD1	12.95	0.10	1
2	A	0	ILE	CG2	17.64	0.10	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
2	A	0	ILE	N	123.17	0.10	1
2	A	60	ARG	H	7.61	0.01	1
2	A	60	ARG	HA	4.11	0.01	1
2	A	60	ARG	HB2	1.71	0.01	2
2	A	60	ARG	HB3	1.55	0.01	2
2	A	60	ARG	HD2	3.13	0.01	1
2	A	60	ARG	HD3	3.13	0.01	1
2	A	60	ARG	HE	7.15	0.01	1
2	A	60	ARG	HG2	1.43	0.01	1
2	A	60	ARG	HG3	1.43	0.01	1
2	A	60	ARG	CA	57.03	0.10	1
2	A	60	ARG	CD	43.19	0.10	1
2	A	60	ARG	CZ	159.53	0.10	1
2	A	60	ARG	N	124.96	0.10	1
2	A	60	ARG	NE	84.66	0.10	1

7.2.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	65	0.17 ± 0.21	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	11	—	None (insufficient data)
$^{13}C'$	61	0.03 ± 0.09	None needed (< 0.5 ppm)
^{15}N	61	0.52 ± 0.40	None needed (imprecise)

7.2.3 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 75%, i.e. 561 atoms were assigned a chemical shift out of a possible 751. 0 out of 8 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	275/278 (99%)	113/113 (100%)	109/112 (97%)	53/53 (100%)
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Aromatic	28/97 (29%)	24/47 (51%)	2/47 (4%)	2/3 (67%)
Overall	561/751 (75%)	351/403 (87%)	151/282 (54%)	59/66 (89%)

7.2.4 Statistically unusual chemical shifts (i)

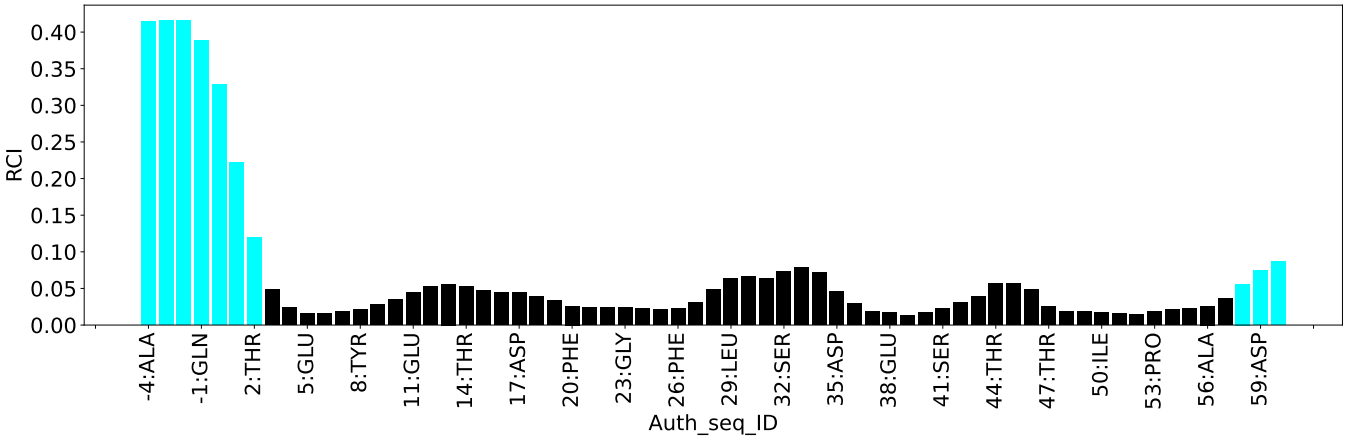
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
2	A	52	SER	HB3	1.59	2.49 – 5.20	-8.3
2	A	52	SER	HB2	1.97	2.61 – 5.13	-7.5
2	A	31	SER	HB3	2.19	2.49 – 5.20	-6.1
2	A	10	TYR	HB3	0.56	0.93 – 4.76	-6.0

7.2.5 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:



7.3 Chemical shift list 3

File name: working_cs.cif

Chemical shift list name: *assignments_fynsh3a39vn53pv55l_3*

7.3.1 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	664
Number of shifts mapped to atoms	588
Number of unparsed shifts	0
Number of shifts with mapping errors	76
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	4

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

- No matching atom found in the structure. First 5 (of 76) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
3	A	-4	ALA	HA	4.32	0.01	1
3	A	-4	ALA	HB1	1.35	0.01	1
3	A	-4	ALA	HB2	1.35	0.01	1
3	A	-4	ALA	HB3	1.35	0.01	1
3	A	-4	ALA	C	177.62	0.10	1
3	A	-4	ALA	CA	52.26	0.10	1
3	A	-4	ALA	CB	19.47	0.10	1
3	A	-3	MET	H	8.49	0.01	1
3	A	-3	MET	HA	4.45	0.01	1
3	A	-3	MET	HB2	2.0	0.01	2
3	A	-3	MET	HB3	2.0	0.01	2
3	A	-3	MET	HE1	2.05	0.01	1
3	A	-3	MET	HE2	2.05	0.01	1
3	A	-3	MET	HE3	2.05	0.01	1
3	A	-3	MET	C	176.08	0.10	1
3	A	-3	MET	CA	55.36	0.10	1
3	A	-3	MET	CE	16.99	0.10	1
3	A	-3	MET	N	120.72	0.10	1
3	A	-2	VAL	H	8.19	0.01	1
3	A	-2	VAL	HA	4.07	0.01	1
3	A	-2	VAL	HB	1.99	0.01	1
3	A	-2	VAL	HG11	0.86	0.01	1
3	A	-2	VAL	HG12	0.86	0.01	1
3	A	-2	VAL	HG13	0.86	0.01	1
3	A	-2	VAL	HG21	0.87	0.01	1
3	A	-2	VAL	HG22	0.87	0.01	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
3	A	-2	VAL	HG23	0.87	0.01	1
3	A	-2	VAL	C	175.75	0.10	1
3	A	-2	VAL	CA	62.12	0.10	1
3	A	-2	VAL	CG1	21.18	0.10	1
3	A	-2	VAL	CG2	20.62	0.10	1
3	A	-2	VAL	N	122.74	0.10	1
3	A	-1	GLN	H	8.51	0.01	1
3	A	-1	GLN	HA	4.34	0.01	1
3	A	-1	GLN	HB2	1.96	0.01	2
3	A	-1	GLN	HB3	1.96	0.01	2
3	A	-1	GLN	HE21	7.51	0.01	2
3	A	-1	GLN	HE22	6.83	0.01	2
3	A	-1	GLN	HG2	2.3	0.01	2
3	A	-1	GLN	HG3	2.3	0.01	2
3	A	-1	GLN	C	175.88	0.10	1
3	A	-1	GLN	CA	55.5	0.10	1
3	A	-1	GLN	CD	180.37	0.10	1
3	A	-1	GLN	CG	33.4	0.10	1
3	A	-1	GLN	N	124.92	0.10	1
3	A	-1	GLN	NE2	112.61	0.10	1
3	A	0	ILE	H	8.33	0.01	1
3	A	0	ILE	HA	4.18	0.01	1
3	A	0	ILE	HB	1.85	0.01	1
3	A	0	ILE	HD11	0.75	0.01	1
3	A	0	ILE	HD12	0.75	0.01	1
3	A	0	ILE	HD13	0.75	0.01	1
3	A	0	ILE	HG12	1.42	0.01	2
3	A	0	ILE	HG13	1.16	0.01	2
3	A	0	ILE	HG21	0.94	0.01	1
3	A	0	ILE	HG22	0.94	0.01	1
3	A	0	ILE	HG23	0.94	0.01	1
3	A	0	ILE	C	176.39	0.10	1
3	A	0	ILE	CA	61.26	0.10	1
3	A	0	ILE	CD1	12.95	0.10	1
3	A	0	ILE	CG2	17.64	0.10	1
3	A	0	ILE	N	123.17	0.10	1
3	A	60	ARG	H	7.61	0.01	1
3	A	60	ARG	HA	4.11	0.01	1
3	A	60	ARG	HB2	1.71	0.01	2
3	A	60	ARG	HB3	1.55	0.01	2
3	A	60	ARG	HD2	3.13	0.01	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
3	A	60	ARG	HD3	3.13	0.01	1
3	A	60	ARG	HE	7.15	0.01	1
3	A	60	ARG	HG2	1.43	0.01	1
3	A	60	ARG	HG3	1.43	0.01	1
3	A	60	ARG	CA	57.03	0.10	1
3	A	60	ARG	CD	43.19	0.10	1
3	A	60	ARG	CZ	159.53	0.10	1
3	A	60	ARG	N	124.96	0.10	1
3	A	60	ARG	NE	84.66	0.10	1

7.3.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	65	0.16 ± 0.10	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	11	—	None (insufficient data)
$^{13}C'$	61	0.01 ± 0.17	None needed (< 0.5 ppm)
^{15}N	61	0.54 ± 0.67	None needed (imprecise)

7.3.3 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 75%, i.e. 561 atoms were assigned a chemical shift out of a possible 751. 0 out of 8 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	275/278 (99%)	113/113 (100%)	109/112 (97%)	53/53 (100%)
Sidechain	258/376 (69%)	214/243 (88%)	40/123 (33%)	4/10 (40%)
Aromatic	28/97 (29%)	24/47 (51%)	2/47 (4%)	2/3 (67%)
Overall	561/751 (75%)	351/403 (87%)	151/282 (54%)	59/66 (89%)

7.3.4 Statistically unusual chemical shifts (i)

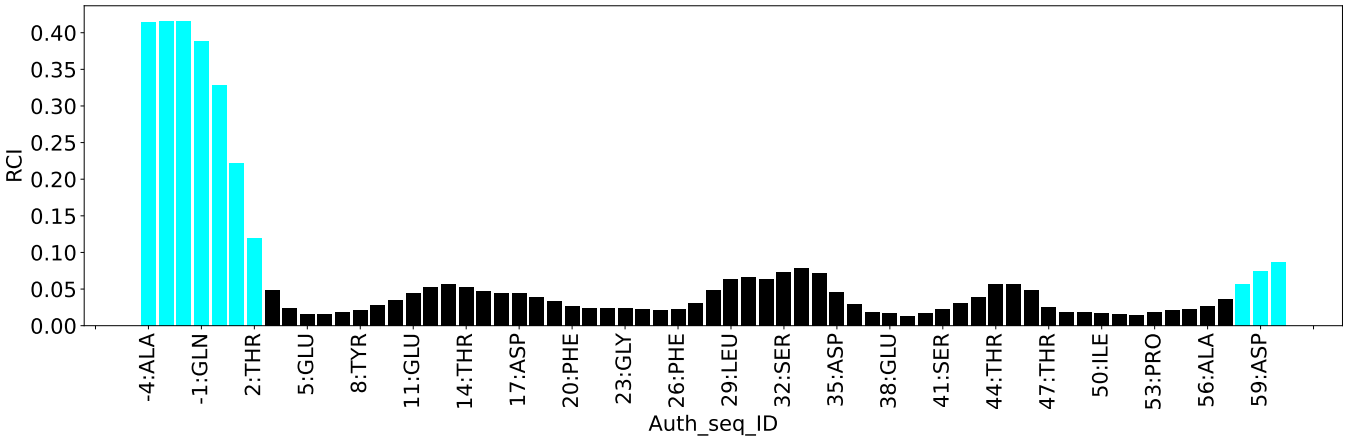
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
3	A	52	SER	HB3	1.59	2.49 – 5.20	-8.3
3	A	52	SER	HB2	1.97	2.61 – 5.13	-7.5
3	A	31	SER	HB3	2.19	2.49 – 5.20	-6.1
3	A	10	TYR	HB3	0.56	0.93 – 4.76	-6.0

7.3.5 Random Coil Index (RCI) plots

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:



7.4 Chemical shift list 4

File name: working_cs.cif

Chemical shift list name: *assignments_fynsh3a39vn53pv55l_4*

7.4.1 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	277
Number of shifts mapped to atoms	277
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

7.4.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	54	0.06 ± 0.11	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	3	—	None (insufficient data)
$^{13}C'$	52	-0.21 ± 0.24	None needed (< 0.5 ppm)
^{15}N	51	0.19 ± 0.35	None needed (< 0.5 ppm)

7.4.3 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 35%, i.e. 266 atoms were assigned a chemical shift out of a possible 751. 0 out of 8 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	237/278 (85%)	87/113 (77%)	101/112 (90%)	49/53 (92%)
Sidechain	25/376 (7%)	0/243 (0%)	25/123 (20%)	0/10 (0%)
Aromatic	4/97 (4%)	2/47 (4%)	0/47 (0%)	2/3 (67%)
Overall	266/751 (35%)	89/403 (22%)	126/282 (45%)	51/66 (77%)

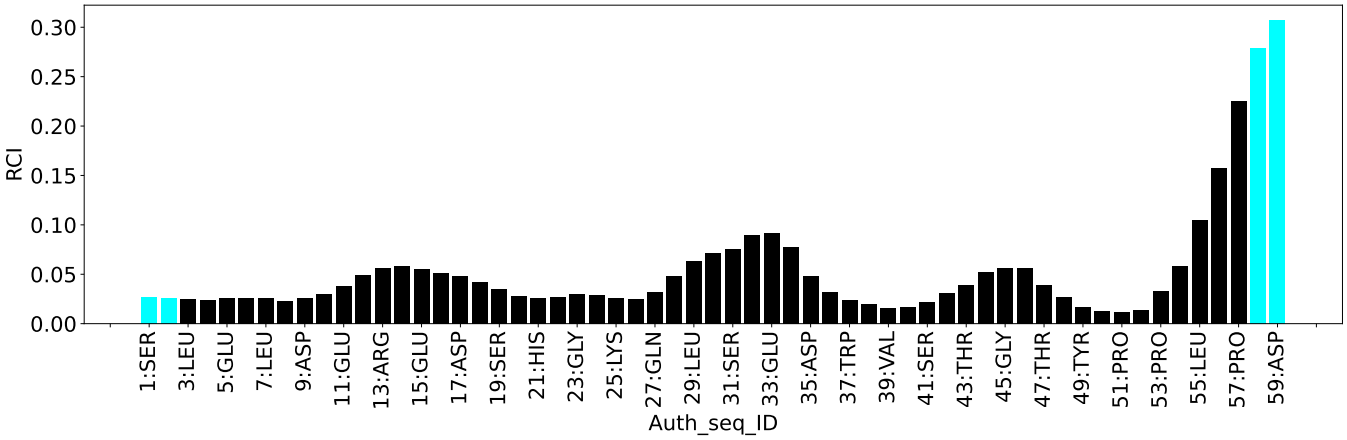
7.4.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

7.4.5 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:


RCI
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1:SER
3:LEU
5:GLU
7:LEU
9:ASP
11:GLU
13:ARG
15:GLU
17:ASP
19:SER
21:HIS
23:GLY
25:LYS
27:GLN
29:LEU
31:SER
33:GLU
35:ASP
37:TRP
39:VAL
41:SER
43:THR
45:GLY
47:THR
49:TYR
51:PRO
53:PRO
55:LEU
57:PRO
59:ASP
Auth_seq_ID

8 NMR restraints analysis

8.1 Conformationally restricting restraints

The following table provides the summary of experimentally observed NMR restraints in different categories. Restraints are classified into different categories based on the sequence separation of the atoms involved.

Description	Value
Total distance restraints	511
Intra-residue (|i-j|=0)	143
Sequential (|i-j|=1)	205
Medium range (|i-j|>1 and |i-j|<5)	49
Long range (|i-j|≥5)	114
Inter-chain	0
Hydrogen bond restraints	0
Disulfide bond restraints	0
Total dihedral-angle restraints	0
Number of unmapped restraints	0
Number of restraints per residue	7.7
Number of long range restraints per residue[1]	1.7

[1]Long range hydrogen bonds and disulfide bonds are counted as long range restraints while calculating the number of long range restraints per residue

8.2 Residual restraint violations

This section provides the overview of the restraint violations analysis. The violations are binned as small, medium and large violations based on its absolute value. Average number of violations per model is calculated by dividing the total number of violations in each bin by the size of the ensemble.

8.2.1 Average number of distance violations per model

Distance violations less than 0.1 Å are not included in the calculation.

Bins (Å)	Average number of violations per model	Max (Å)
0.1-0.2 (Small)	8.4	0.2
0.2-0.5 (Medium)	18.8	0.5
>0.5 (Large)	27.7	2.13

8.2.2 Average number of dihedral-angle violations per model (i)

Dihedral-angle violations less than 1° are not included in the calculation.There are no dihedral-angle violations

9 Distance violation analysis (i)

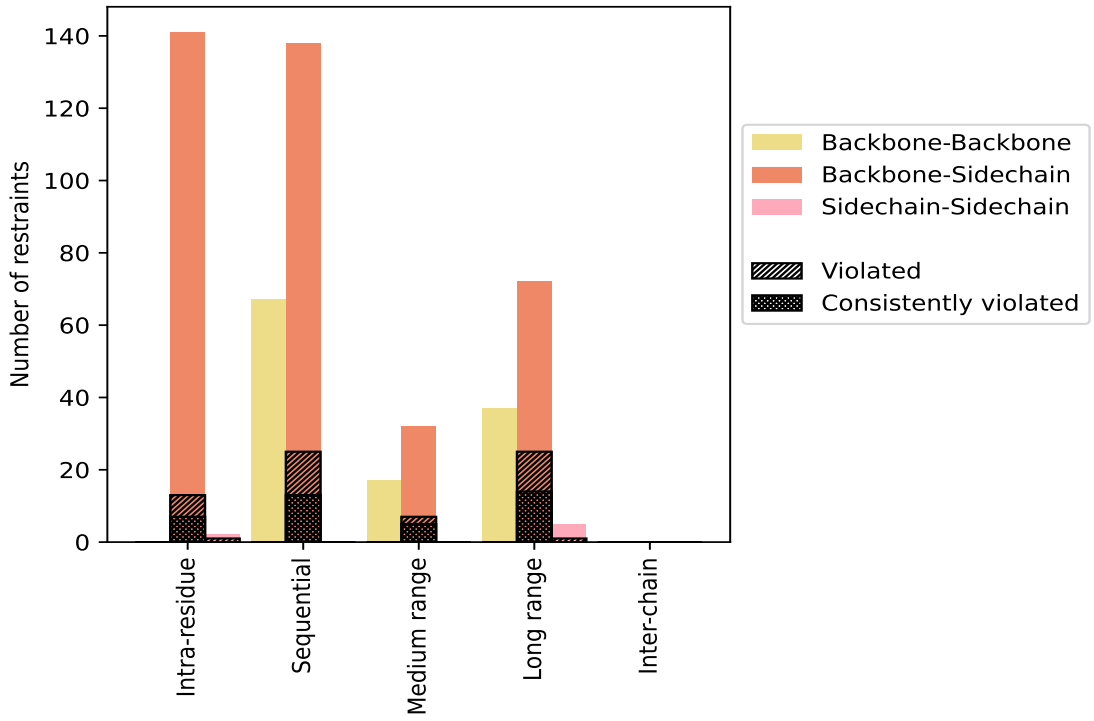
9.1 Summary of distance violations (i)

The following table shows the summary of distance violations in different restraint categories based on the sequence separation of the atoms involved. Each category is further sub-divided into three sub-categories based on the atoms involved. Violations less than 0.1 Å are not included in the statistics.

Restraints type	Count	%[1]	Violated[3]			Consistently Violated[4]		
			Count	%[2]	%[1]	Count	%[2]	%[1]
Intra-residue (|i-j|=0)	143	28.0	14	9.8	2.7	7	4.9	1.4
Backbone-Backbone	0	0.0	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	141	27.6	13	9.2	2.5	7	5.0	1.4
Sidechain-Sidechain	2	0.4	1	50.0	0.2	0	0.0	0.0
Sequential (|i-j|=1)	205	40.1	25	12.2	4.9	13	6.3	2.5
Backbone-Backbone	67	13.1	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	138	27.0	25	18.1	4.9	13	9.4	2.5
Sidechain-Sidechain	0	0.0	0	0.0	0.0	0	0.0	0.0
Medium range (|i-j|>1 & |i-j|<5)	49	9.6	7	14.3	1.4	5	10.2	1.0
Backbone-Backbone	17	3.3	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	32	6.3	7	21.9	1.4	5	15.6	1.0
Sidechain-Sidechain	0	0.0	0	0.0	0.0	0	0.0	0.0
Long range (|i-j|≥5)	114	22.3	26	22.8	5.1	14	12.3	2.7
Backbone-Backbone	37	7.2	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	72	14.1	25	34.7	4.9	14	19.4	2.7
Sidechain-Sidechain	5	1.0	1	20.0	0.2	0	0.0	0.0
Inter-chain	0	0.0	0	0.0	0.0	0	0.0	0.0
Backbone-Backbone	0	0.0	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	0	0.0	0	0.0	0.0	0	0.0	0.0
Sidechain-Sidechain	0	0.0	0	0.0	0.0	0	0.0	0.0
Hydrogen bond	0	0.0	0	0.0	0.0	0	0.0	0.0
Disulfide bond	0	0.0	0	0.0	0.0	0	0.0	0.0
Total	511	100.0	72	14.1	14.1	39	7.6	7.6
Backbone-Backbone	121	23.7	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	383	75.0	70	18.3	13.7	39	10.2	7.6
Sidechain-Sidechain	7	1.4	2	28.6	0.4	0	0.0	0.0

[1] percentage calculated with respect to the total number of distance restraints, [2] percentage calculated with respect to the number of restraints in a particular restraint category, [3] violated in at least one model, [4] violated in all the models

9.1.1 Bar chart : Distribution of distance restraints and violations



Violated and consistently violated restraints are shown using different hatch patterns in their respective categories. The hydrogen bonds and disulfied bonds are counted in their appropriate category on the x-axis

9.2 Distance violation statistics for each model

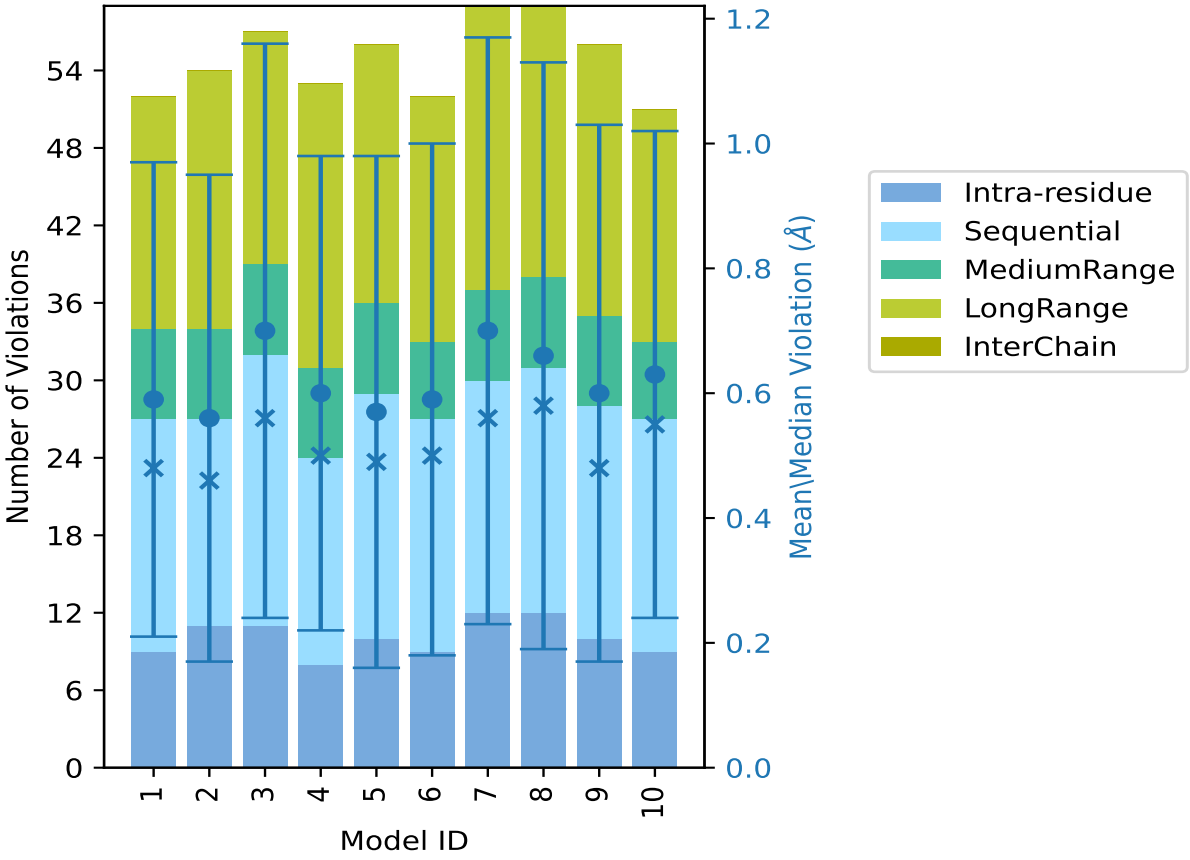
The following table provides the distance violation statistics for each model in the ensemble. Violations less than 0.1 Å are not included in the statistics.

Model ID	Number of violations						Mean (Å)	Max (Å)	SD[6] (Å)	Median (Å)
	IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total				
1	9	18	7	18	0	52	0.59	1.74	0.38	0.48
2	11	16	7	20	0	54	0.56	1.78	0.39	0.46
3	11	21	7	18	0	57	0.7	1.99	0.46	0.56
4	8	16	7	22	0	53	0.6	1.77	0.38	0.5
5	10	19	7	20	0	56	0.57	2.0	0.41	0.49
6	9	18	6	19	0	52	0.59	1.79	0.41	0.5
7	12	18	7	22	0	59	0.7	2.05	0.47	0.56
8	12	19	7	21	0	59	0.66	2.02	0.47	0.58
9	10	18	7	21	0	56	0.6	2.13	0.43	0.48
10	9	18	6	18	0	51	0.63	1.73	0.39	0.55

[1]Intra-residue restraints, [2]Sequential restraints, [3]Medium range restraints, [4]Long range restraints,

[5]Inter-chain restraints, [6]Standard deviation

9.2.1 Bar graph : Distance Violation statistics for each model



The mean(dot),median(x) and the standard deviation are shown in blue with respect to the y axis on the right

9.3 Distance violation statistics for the ensemble

Violation analysis may find that some restraints are violated in few models and some are violated in most of models. The following table provides this information as number of violated restraints for a given fraction of the ensemble. In total, 439(IR:129, SQ:180, MR:42, LR:88, IC:0) restraints are not violated in the ensemble.

Number of violated restraints						Fraction of the ensemble	
IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total	Count[6]	%
1	2	0	1	0	4	1	10.0
1	2	0	1	0	4	2	20.0
1	3	0	4	0	8	3	30.0
1	1	0	0	0	2	4	40.0

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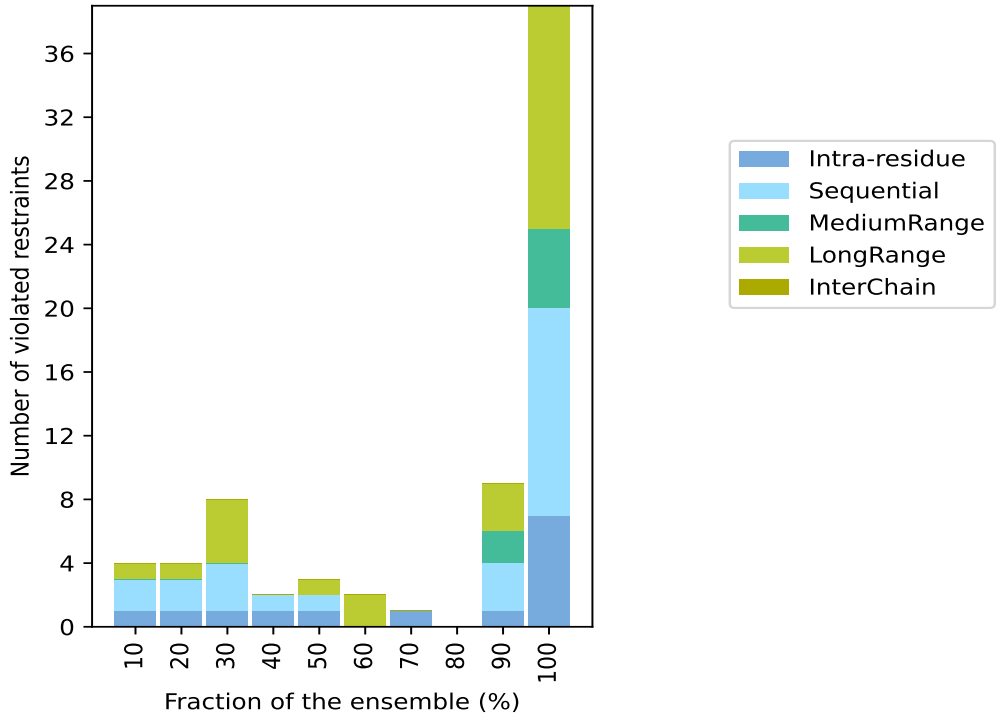
Number of violated restraints						Fraction of the ensemble	
IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total	Count[6]	%
1	1	0	1	0	3	5	50.0
0	0	0	2	0	2	6	60.0
1	0	0	0	0	1	7	70.0
0	0	0	0	0	0	8	80.0
1	3	2	3	0	9	9	90.0
7	13	5	14	0	39	10	100.0

[1]Intra-residue restraints, [2]Sequential restraints, [3]Medium range restraints, [4]Long range restraints, [5]Inter-chain restraints, [6] Number of models with violations

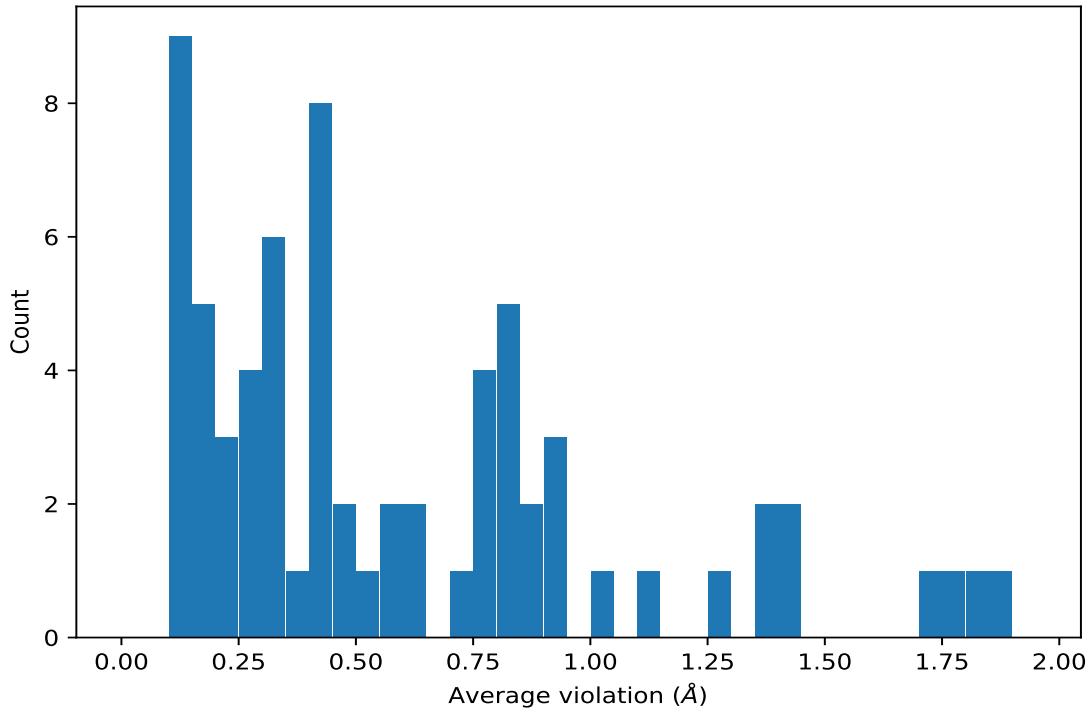
9.3.1 Bar graph : Distance violation statistics for the ensemble



9.4 Most violated distance restraints in the ensemble

9.4.1 Histogram : Distribution of mean distance violations

The following histogram shows the distribution of the average value of the violation. The average is calculated for each restraint that is violated in more than one model over all the violated models in the ensemble



9.4.2 Table: Most violated distance restraints

The following table provides the mean and the standard deviation of the violations for the 10 worst performing restraints, sorted by number of violated models and the mean violation value. The Key (restraint list ID, restraint ID) is the unique identifier for a given restraint. Rows with same key represent combinatorial or ambiguous restraints and are counted as a single restraint.

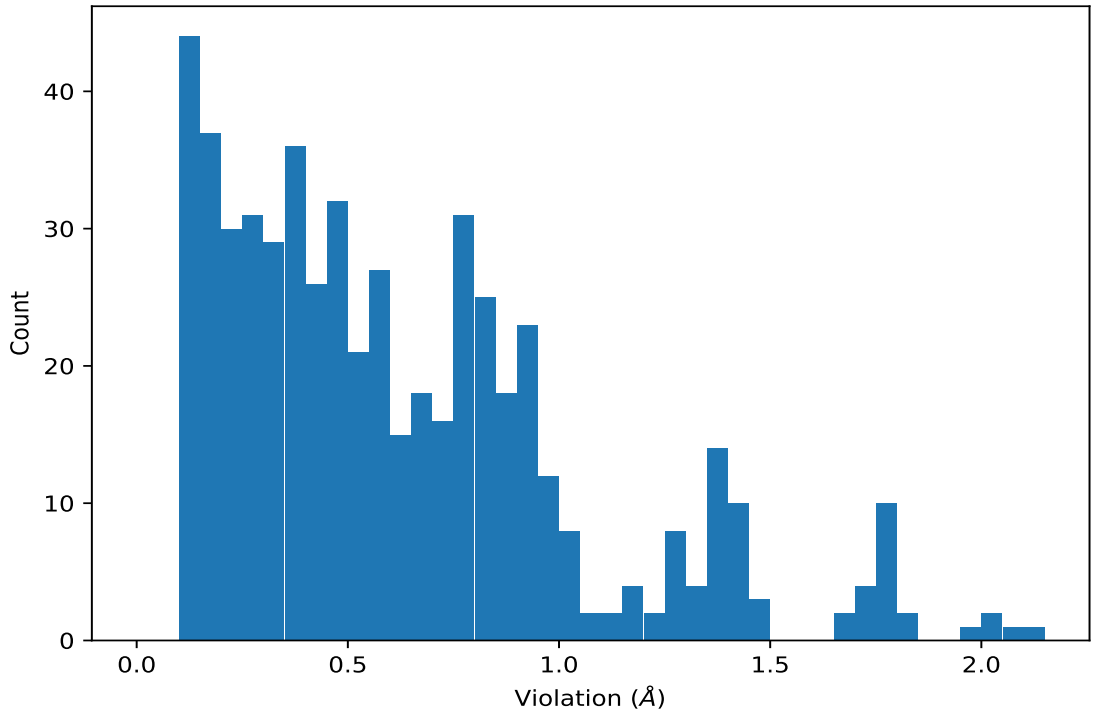
Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(1,279)	1:A:6:ALA:H	1:A:55:LEU:HB2	10	1.76	0.03	1.76
(1,242)	1:A:7:LEU:H	1:A:8:TYR:HD1	10	1.74	0.24	1.76
(1,254)	1:A:44:THR:HG21	1:A:45:GLY:H	10	1.39	0.01	1.38
(1,211)	1:A:12:ALA:HB1	1:A:20:PHE:H	10	1.38	0.05	1.38
(1,216)	1:A:43:THR:H	1:A:44:THR:HG21	10	1.27	0.03	1.27
(1,343)	1:A:41:SER:HB2	1:A:45:GLY:H	10	1.03	0.18	0.97
(1,366)	1:A:12:ALA:HB1	1:A:14:THR:H	10	0.94	0.05	0.94
(1,259)	1:A:47:THR:HG21	1:A:48:GLY:H	10	0.94	0.03	0.93
(1,316)	1:A:27:GLN:HB2	1:A:40:ARG:H	10	0.9	0.11	0.92
(1,206)	1:A:10:TYR:HB2	1:A:20:PHE:H	10	0.86	0.09	0.87

[1]Number of violated models, [2]Standard deviation

9.5 All violated distance restraints (i)

9.5.1 Histogram : Distribution of distance violations (i)

The following histogram shows the distribution of the absolute value of the violation for all violated restraints in the ensemble.



9.5.2 Table : All distance violations (i)

The following table provides the 10 worst performing restraints, sorted by the violation value. The Key (restraint list ID, restraint ID) is the unique identifier for a given restraint. Rows with same key represent combinatorial or ambiguous restraints and are counted as a single restraint.

Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,242)	1:A:7:LEU:H	1:A:8:TYR:HD1	9	2.13
(1,160)	1:A:26:PHE:HD1	1:A:40:ARG:H	7	2.05
(1,242)	1:A:7:LEU:H	1:A:8:TYR:HD1	8	2.02
(1,242)	1:A:7:LEU:H	1:A:8:TYR:HD1	5	2.0
(1,93)	1:A:4:PHE:H	1:A:26:PHE:HD2	3	1.99
(1,160)	1:A:26:PHE:HD1	1:A:40:ARG:H	3	1.81
(1,279)	1:A:6:ALA:H	1:A:55:LEU:HB2	5	1.8
(1,242)	1:A:7:LEU:H	1:A:8:TYR:HD1	6	1.79
(1,93)	1:A:4:PHE:H	1:A:26:PHE:HD2	7	1.78
(1,279)	1:A:6:ALA:H	1:A:55:LEU:HB2	2	1.78

10 Dihedral-angle violation analysis (i)

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