



wwPDB NMR Structure Validation Summary Report ⓘ

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PDB ID : 2KZA
BMRB ID : 17001
Title : Solution structure of ASIP(80-132, P103A, P105A, Q115Y, S124Y)
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This is a wwPDB NMR Structure Validation Summary Report for a publicly released PDB entry.

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A user guide is available at

<https://www.wwpdb.org/validation/2017/NMRValidationReportHelp>

with specific help available everywhere you see the ⓘ 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 ⓘ](#)) 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) : 2.37.1

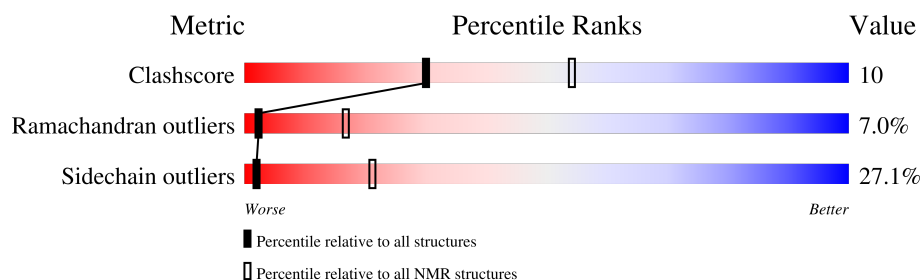
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

SOLUTION NMR

The overall completeness of chemical shifts assignment is 48%.

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 $\leq 5\%$

Mol	Chain	Length	Quality of chain
1	A	53	

2 Ensemble composition and analysis

This entry contains 20 models. Model 7 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:93-A:132 (40)	0.55	7

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 5 single-model clusters were found.

Cluster number	Models
1	1, 4, 5, 7, 8, 9, 10, 11, 12, 15, 17, 18, 19
2	2, 3
Single-model clusters	6; 13; 14; 16; 20

3 Entry composition

There is only 1 type of molecule in this entry. The entry contains 566 atoms, of which 273 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Agouti-signaling protein.

Mol	Chain	Residues	Atoms						Trace
1	A	40	Total	C	H	N	O	S	0
			566	177	273	55	51	10	

There are 4 discrepancies between the modelled and reference sequences:

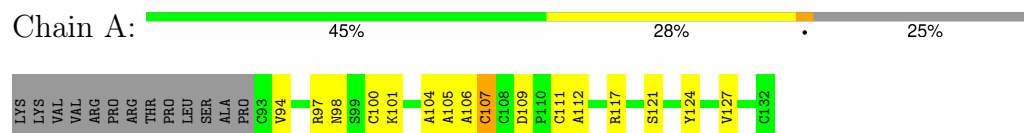
Chain	Residue	Modelled	Actual	Comment	Reference
A	103	ALA	PRO	engineered mutation	UNP P42127
A	105	ALA	PRO	engineered mutation	UNP P42127
A	115	TYR	GLN	engineered mutation	UNP P42127
A	124	TYR	SER	engineered mutation	UNP P42127

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.

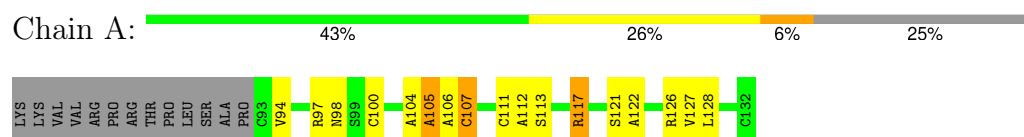
- Molecule 1: Agouti-signaling protein



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

The representative model is number 7. Colouring as in section 4.1 above.

- Molecule 1: Agouti-signaling protein



5 Refinement protocol and experimental data overview

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

Of the 100 calculated structures, 20 were deposited, based on the following criterion: *target function*.

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

Software name	Classification	Version
CYANA	refinement	2.1
CYANA	structure solution	2.1

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	303
Number of shifts mapped to atoms	235
Number of unparsed shifts	0
Number of shifts with mapping errors	68
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	48%

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	293	273	272	6±2
All	All	5860	5460	5440	113

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 10.

5 of 27 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:100:CYS:O	1:A:101:LYS:C	0.67	2.34	1	5
1:A:94:VAL:HG23	1:A:106:ALA:HB1	0.60	1.74	4	12
1:A:112:ALA:HB2	1:A:127:VAL:HG12	0.60	1.73	4	9
1:A:94:VAL:HG21	1:A:99:SER:O	0.60	1.97	14	1
1:A:104:ALA:O	1:A:105:ALA:HB3	0.59	1.98	9	13

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	38/53 (72%)	24±1 (62±3%)	12±2 (31±4%)	3±1 (7±3%)	2	17
All	All	760/1060 (72%)	470 (62%)	237 (31%)	53 (7%)	2	17

5 of 7 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	A	107	CYS	19
1	A	105	ALA	16
1	A	108	CYS	8
1	A	109	ASP	5
1	A	101	LYS	2

6.3.2 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 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	33/45 (73%)	24±2 (73±6%)	9±2 (27±6%)	2	21
All	All	660/900 (73%)	481 (73%)	179 (27%)	2	21

5 of 21 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	97	ARG	20
1	A	117	ARG	20
1	A	121	SER	20
1	A	107	CYS	19
1	A	101	LYS	16

6.3.3 RNA ⓘ

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 48% for the well-defined parts and 48% 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

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	303
Number of shifts mapped to atoms	235
Number of unparsed shifts	0
Number of shifts with mapping errors	68
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	2

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 68) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	80	LYS	H	8.196	0.003	1
1	A	80	LYS	HA	4.168	0.003	1
1	A	80	LYS	HB2	1.703	0.001	2
1	A	80	LYS	HB3	1.703	0.001	2
1	A	80	LYS	HG2	1.629	0.008	2
1	A	80	LYS	HG3	1.629	0.008	2
1	A	82	VAL	H	8.216	0.004	1
1	A	82	VAL	HA	4.024	0.003	1
1	A	82	VAL	HB	1.933	0.011	1
1	A	82	VAL	HG11	0.821	0.000	.
1	A	82	VAL	HG12	0.821	0.000	.
1	A	82	VAL	HG13	0.821	0.000	.
1	A	82	VAL	HG21	0.862	0.000	.
1	A	82	VAL	HG22	0.862	0.000	.

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	82	VAL	HG23	0.862	0.000	.
1	A	83	VAL	H	8.336	0.006	1
1	A	83	VAL	HA	4.03	0.009	1
1	A	83	VAL	HB	1.934	0.003	1
1	A	83	VAL	HG11	0.819	0.000	.
1	A	83	VAL	HG12	0.819	0.000	.
1	A	83	VAL	HG13	0.819	0.000	.
1	A	83	VAL	HG21	0.856	0.001	.
1	A	83	VAL	HG22	0.856	0.001	.
1	A	83	VAL	HG23	0.856	0.001	.
1	A	84	ARG	H	8.443	0.005	1
1	A	84	ARG	HA	4.575	0.001	1
1	A	84	ARG	HB2	1.78	0.004	2
1	A	84	ARG	HB3	1.655	0.003	2
1	A	84	ARG	HG2	1.578	0.004	2
1	A	84	ARG	HG3	1.612	0.007	2
1	A	85	PRO	HA	4.357	0.004	1
1	A	85	PRO	HB2	1.836	0.003	2
1	A	85	PRO	HB3	2.228	0.008	2
1	A	86	ARG	H	8.474	0.004	1
1	A	86	ARG	HA	4.262	0.005	1
1	A	86	ARG	HB2	1.709	0.002	2
1	A	86	ARG	HB3	1.767	0.006	2
1	A	86	ARG	HG2	1.557	0.016	2
1	A	86	ARG	HG3	1.615	0.006	2
1	A	87	THR	H	8.186	0.005	1
1	A	87	THR	HA	4.353	0.004	1
1	A	87	THR	HB	3.773	0.006	1
1	A	87	THR	HG21	1.296	0.005	.
1	A	87	THR	HG22	1.296	0.005	.
1	A	87	THR	HG23	1.296	0.005	.
1	A	88	PRO	HA	4.345	0.006	1
1	A	88	PRO	HB2	1.84	0.007	2
1	A	88	PRO	HB3	2.245	0.008	2
1	A	89	LEU	H	8.278	0.005	1
1	A	89	LEU	HA	4.273	0.003	1
1	A	89	LEU	HB2	1.575	0.003	2
1	A	89	LEU	HB3	1.575	0.003	2
1	A	89	LEU	HD11	0.827	0.004	.
1	A	89	LEU	HD12	0.827	0.004	.
1	A	89	LEU	HD13	0.827	0.004	.

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	89	LEU	HD21	0.867	0.000	.
1	A	89	LEU	HD22	0.867	0.000	.
1	A	89	LEU	HD23	0.867	0.000	.
1	A	89	LEU	HG	1.534	0.007	1
1	A	90	SER	H	8.205	0.009	1
1	A	91	ALA	H	8.178	0.003	1
1	A	91	ALA	HA	4.545	0.006	1
1	A	91	ALA	HB1	1.177	0.005	.
1	A	91	ALA	HB2	1.177	0.005	.
1	A	91	ALA	HB3	1.177	0.005	.
1	A	92	PRO	HA	4.398	0.009	1
1	A	92	PRO	HB2	1.858	0.006	2
1	A	92	PRO	HB3	2.239	0.003	2

7.1.2 Chemical shift referencing [i](#)

No chemical shift referencing corrections were calculated (not enough data).

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 48%, i.e. 235 atoms were assigned a chemical shift out of a possible 489. 0 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	¹ H	¹³ C	¹⁵ N
Backbone	78/196 (40%)	78/78 (100%)	0/80 (0%)	0/38 (0%)
Sidechain	141/255 (55%)	141/167 (84%)	0/73 (0%)	0/15 (0%)
Aromatic	16/38 (42%)	16/18 (89%)	0/20 (0%)	0/0 (—%)
Overall	235/489 (48%)	235/263 (89%)	0/173 (0%)	0/53 (0%)

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	117	ARG	HD2	1.61	1.97 – 4.26	-6.6
1	A	117	ARG	HD3	1.61	1.81 – 4.39	-5.8

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:

