

# Full wwPDB NMR Structure Validation Report (i)

## Oct 17, 2021 – 10:00 AM EDT

PDB ID	:	1KCY
Title	:	NMR solution structure of apo calbindin D9k (F36G + P43M mutant)
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Deposited on	:	2001-11-12

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

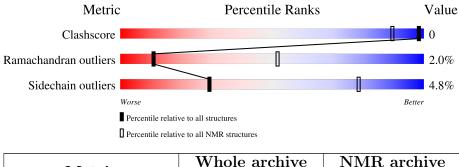
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. $(2010)$
ShiftChecker	:	2.23.2
Ideal geometry (proteins)	:	Engh & Huber $(2001)$
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.23.2

# 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 was not calculated.

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)	(#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	٨	I			
	A	75	92%	•	•



# 2 Ensemble composition and analysis (i)

This entry contains 22 models. Model 19 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 mode							
1	A:1-A:72 (72)	0.40	19				

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 3 clusters and 1 single-model cluster was found.

Cluster number	Models
1	$\begin{array}{c}1, \ 3, \ 4, \ 5, \ 6, \ 8, \ 10, \ 11, \ 12, \ 13, \ 14, \ 15, \ 16, \ 18, \ 19, \\21, \ 22\end{array}$
2	17, 20
3	2, 9
Single-model clusters	7



# 3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 1192 atoms, of which 598 are hydrogens and 0 are deuteriums.

• Molecule 1 is a protein called calbindin D9k.

Mol	Chain	Residues		L	Atom	IS			Trace
1	Δ	75	Total	С	Η	Ν	0	S	0
	A	75	1192	377	598	91	125	1	0

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
А	36	GLY	PHE	engineered mutation	UNP P02633
А	43	MET	PRO	engineered mutation	UNP P02633

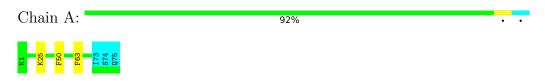


# 4 Residue-property plots (i)

# 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.

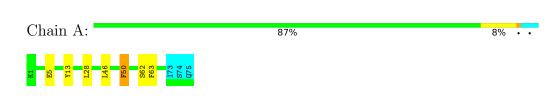
• Molecule 1: calbindin D9k



# 4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

## 4.2.1 Score per residue for model 1



## 4.2.2 Score per residue for model 2

• Molecule 1: calbindin D9k

• Molecule 1: calbindin D9k

Chain A: 85% 11% .



## 4.2.3 Score per residue for model 3

• Molecule 1: calbindin D9k

Chain A:	84%	12%	•
K1 K25 K25 C132 Q33 Q33 C50 C59 C59 C59 C59 C59 C59 C59 C59 C59 C59			

#### 4.2.4 Score per residue for model 4

• Molecule 1: calbindin D9k

Chain A:	87%	8%	•••
K1 K2 E55 E51 C59 C59 C59 C59 C59 C59 C59 C59 C59 C59			

#### 4.2.5 Score per residue for model 5

• Molecule 1: calbindin D9k

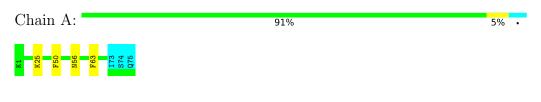
Cl	nai	n A	<b>\</b> :		_		91%	 5%	·
K1	E35	S44	N56	<mark>G59</mark>	173 S74	Q75			

#### 4.2.6 Score per residue for model 6

• Molecule 1: calbindin D9k

Chain A: 91% 5% •

#### 4.2.7 Score per residue for model 7





## 4.2.8 Score per residue for model 8

• Molecule 1: calbindin D9k

Chain A:	91%	5% •
KI F60 844 773 874 773 775		

#### 4.2.9 Score per residue for model 9

• Molecule 1: calbindin D9k

Chain A:	88%	7%	•	·
K1 Y13 K16 K25 M43 S44 F50	173 874 775			

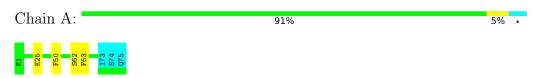
#### 4.2.10 Score per residue for model 10

• Molecule 1: calbindin D9k

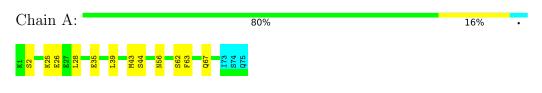
С	ha	in	A	<b>\</b> :								88%	7	% •	• •
K1	S24	0	821 K20	L30	E35	_	F50	це с	I73	S74	Q75				

#### 4.2.11 Score per residue for model 11

• Molecule 1: calbindin D9k



## 4.2.12 Score per residue for model 12





## 4.2.13 Score per residue for model 13

• Molecule 1: calbindin D9k

Chain A:	91%	5% •
K1 Y13 S44 F50 F50 S74 G75 G75		

#### 4.2.14 Score per residue for model 14

• Molecule 1: calbindin D9k

C	hain A:		89%	5% • •
K1	844 145 146 146 146 146 150	562 I 73 S 74 Q 75		

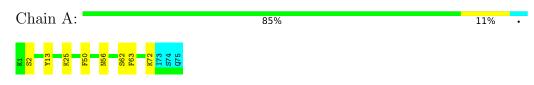
### 4.2.15 Score per residue for model 15

• Molecule 1: calbindin D9k

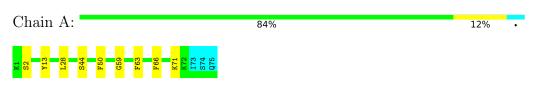
$\mathrm{Ch}$	air	n A	4:						88%	8%	•
K1	L30	L46	F50	N56	G57	F63	 I73 S74	Q75			

#### 4.2.16 Score per residue for model 16

• Molecule 1: calbindin D9k



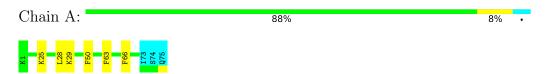
## 4.2.17 Score per residue for model 17





### 4.2.18 Score per residue for model 18

• Molecule 1: calbindin D9k



#### 4.2.19 Score per residue for model 19 (medoid)

• Molecule 1: calbindin D9k

Chain A:	91%	5% •
K1 K29 M43 M43 M43 F50 F50 874 8774 8775		

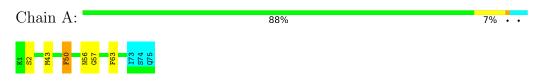
#### 4.2.20 Score per residue for model 20

• Molecule 1: calbindin D9k

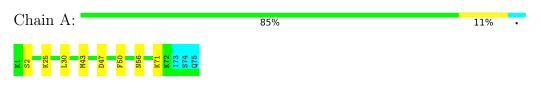
C	ha	ir	1.	A	•							87%	8%	•	•
K1 S	70	K25	E26			001	G59	L CC	173	S74	Q75				

#### 4.2.21 Score per residue for model 21

• Molecule 1: calbindin D9k



#### 4.2.22 Score per residue for model 22





# 5 Refinement protocol and experimental data overview (i)

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

Of the 50 calculated structures, 22 were deposited, based on the following criterion: The full ensemble was ordered by lowest residual constraint violations, then the top 22 with favorable covalent geometries and AMBER energies were selected.

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

Software name	Classification	Version
DIANA	structure solution	2.8
Amber	structure solution	4.1
Amber	refinement	4.1

No chemical shift data was provided.



# 6 Model quality (i)

# 6.1 Standard geometry (i)

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 (average) root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	B	ond lengths	Bond angles			
		RMSZ	#Z > 5	RMSZ	$\#Z{>}5$		
1	А	$0.53 {\pm} 0.01$	$0{\pm}0/578~(~0.0{\pm}~0.0\%)$	$0.84{\pm}0.01$	$0{\pm}1/773~(~0.0{\pm}~0.1\%)$		
All	All	0.53	0/12716~(~0.0%)	0.84	8/17006~(~0.0%)		

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand. A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	Chirality	Planarity
1	А	$0.0{\pm}0.0$	$1.4{\pm}0.8$
All	All	0	31

There are no bond-length outliers.

All unique angle outliers are listed below. They are sorted according to the Z-score of the worst occurrence in the ensemble.

Mol	Chain	Res	Trune	Atoma	7	Observed(°)	$Ideal(^{o})$	Moo	lels
	Chain	nes	Type	Atoms		Observed(*)	Ideal(*)	Worst	Total
1	А	50	PHE	CB-CG-CD1	-5.35	117.05	120.80	20	1
1	А	13	TYR	CB-CG-CD2	-5.28	117.83	121.00	1	6
1	А	50	PHE	CB-CG-CD2	-5.10	117.23	120.80	1	1

There are no chirality outliers.

All unique planar outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Group	Models (Total)
1	А	50	PHE	Sidechain	17
1	А	63	PHE	Sidechain	12
1	А	66	PHE	Sidechain	2



## 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.

Mo	Chain	Non-H	H(model)	H(added)	Clashes
1	А	570	574	574	0±1
All	All	12540	12628	12628	6

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 0.

All 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:28:LEU:HD13	1:A:66:PHE:CE1	0.58	2.33	10	1
1:A:46:LEU:HD12	1:A:46:LEU:H	0.46	1.71	3	3
1:A:28:LEU:C	1:A:28:LEU:HD12	0.45	2.31	10	1
1:A:46:LEU:H	1:A:46:LEU:HD22	0.44	1.72	15	1

## 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	Perce	entiles
1	А	71/75~(95%)	$62\pm2$ (87 $\pm2\%$ )	$8\pm2~(11\pm3\%)$	$1\pm1~(2\pm1\%)$	11	52
All	All	1562/1650~(95%)	1357 (87%)	173 (11%)	32~(2%)	11	52

All 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	А	25	LYS	11
1	А	44	SER	8
1	А	59	GLY	5
1	А	57	GLY	3

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Mol	Chain	Res	Type	Models (Total)
1	А	43	MET	3
1	А	32	LEU	1
1	А	33	GLN	1

### 6.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent side chain 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 side chain conformation was analysed and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Perce	ntiles
1	А	64/67~(96%)	$61\pm2$ (95±3%)	$3\pm2~(5\pm3\%)$	29	78
All	All	1408/1474~(96%)	1340 (95%)	68~(5%)	29	78

All 23 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	А	56	ASN	10
1	А	62	SER	7
1	А	50	PHE	7
1	А	28	LEU	6
1	А	35	GLU	6
1	А	2	SER	6
1	А	43	MET	4
1	А	29	LYS	3
1	А	30	LEU	3
1	А	26	GLU	2
1	А	71	LYS	2
1	А	5	GLU	1
1	А	46	LEU	1
1	А	45	THR	1
1	А	51	GLU	1
1	А	16	LYS	1
1	А	24	SER	1
1	А	39	LEU	1
1	А	67	GLN	1
1	А	72	LYS	1
1	А	44	SER	1
1	А	49	LEU	1

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Mol	Chain	Res	Type	Models (Total)
1	А	47	ASP	1

## 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)

No chemical shift data were provided

