



wwPDB NMR Structure Validation Summary Report ⓘ

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PDB ID : 2N3Y
Title : NMR structure of the Y48pCMF variant of human cytochrome c in its reduced state
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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

<http://wwpdb.org/validation/2016/NMRValidationReportHelp>

with specific help available everywhere you see the ⓘ symbol.

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

Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	1.7.1 (RC1), CSD as537be (2016)
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	rb-20028442
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	rb-20028442

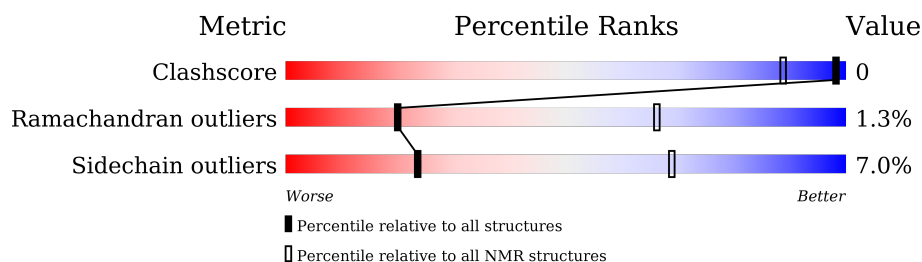
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

SOLUTION NMR

The overall completeness of chemical shifts assignment is 88%.

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	114402	11133
Ramachandran outliers	111179	9975
Sidechain outliers	111093	9958

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	104	

2 Ensemble composition and analysis

This entry contains 20 models. Model 20 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:1-A:40, A:58-A:104 (87)	0.46	20

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 and 5 single-model clusters were found.

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

3 Entry composition [i](#)

There are 2 unique types of molecules in this entry. The entry contains 1738 atoms, of which 876 are hydrogens and 0 are deuteriums.

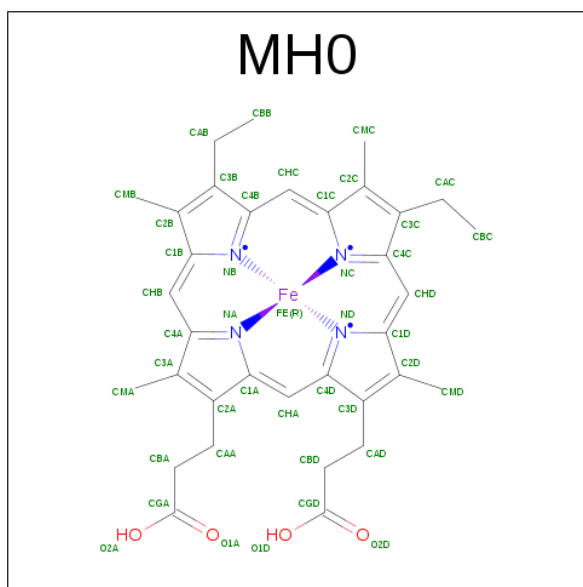
- Molecule 1 is a protein called Cytochrome c.

Mol	Chain	Residues	Atoms						Trace
1	A	104	Total	C	H	N	O	S	0
			1663	523	844	142	149	5	

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	48	1PA	TYR	ENGINEERED MUTATION	UNP P99999

- Molecule 2 is MESOHEME (three-letter code: MH0) (formula: $C_{34}H_{36}FeN_4O_4$).



Mol	Chain	Residues	Atoms					
2	A	1	Total	C	Fe	H	N	O
			75	34	1	32	4	4

5 Refinement protocol and experimental data overview

The models were refined using the following method: *distance geometry, molecular dynamics*.

Of the 200 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	structure solution	2.1
AMBER	refinement	
TALOS	geometry optimization	
CYANA	refinement	

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)	2n3y_cs.cif
Number of chemical shift lists	1
Total number of shifts	1263
Number of shifts mapped to atoms	1242
Number of unparsed shifts	2
Number of shifts with mapping errors	19
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	88%

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

6 Model quality i

6.1 Standard geometry i

Bond lengths and bond angles in the following residue types are not validated in this section: 1PA, MH0

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	Bond lengths		Bond angles	
		RMSZ	#Z>5	RMSZ	#Z>5
1	A	0.65±0.01	0±0/707 (0.0±0.0%)	1.02±0.03	2±1/939 (0.2±0.1%)
All	All	0.65	0/14140 (0.0%)	1.02	36/18780 (0.2%)

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	A	0.0±0.0	0.7±1.1
All	All	0	14

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	Type	Atoms	Z	Observed(°)	Ideal(°)	Models	
								Worst	Total
1	A	38	ARG	NE-CZ-NH1	10.31	125.46	120.30	7	17
1	A	91	ARG	NE-CZ-NH1	9.11	124.85	120.30	8	17
1	A	91	ARG	NH1-CZ-NH2	-5.37	113.50	119.40	5	1
1	A	38	ARG	CD-NE-CZ	5.10	130.74	123.60	12	1

There are no chirality outliers.

5 of 8 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	A	67	TYR	Sidechain	3
1	A	38	ARG	Sidechain,Peptide	3

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Mol	Chain	Res	Type	Group	Models (Total)
1	A	79	LYS	Peptide	2
1	A	91	ARG	Sidechain	2
1	A	103	ASN	Peptide	1

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	694	725	725	1±1
2	A	43	32	32	0±1
All	All	14740	15140	15140	15

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.

5 of 12 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:26:HIS:CD2	1:A:27:LYS:H	0.68	2.06	18	1
1:A:7:LYS:HE3	1:A:97:TYR:CZ	0.52	2.40	7	1
2:A:201:MH0:H29	2:A:201:MH0:CGD	0.50	2.36	4	1
1:A:35:LEU:CD1	2:A:201:MH0:H7	0.47	2.39	17	1
1:A:94:LEU:HD11	2:A:201:MH0:H13	0.46	1.88	6	4

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	85/104 (82%)	76±2 (89±2%)	8±2 (10±2%)	1±1 (1±1%)	20	66
All	All	1700/2080 (82%)	1510 (89%)	168 (10%)	22 (1%)	20	66

5 of 11 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	77	GLY	6
1	A	34	GLY	3
1	A	20	VAL	3
1	A	80	MET	2
1	A	78	THR	2

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	74/84 (88%)	69±2 (93±2%)	5±2 (7±2%)	23 69
All	All	1480/1680 (88%)	1376 (93%)	104 (7%)	23 69

5 of 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	A	72	LYS	20
1	A	70	ASN	20
1	A	32	LEU	13
1	A	58	ILE	12
1	A	73	LYS	8

6.3.3 RNA [i](#)

There are no RNA molecules in this entry.

6.4 Non-standard residues in protein, DNA, RNA chains [i](#)

1 non-standard protein/DNA/RNA residue is modelled in this entry.

In the following table, the Counts columns list the number of bonds for which Mogul statistics could be retrieved, the number of bonds that are observed in the model and the number of bonds that are defined in the chemical component dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond length is the number of standard

deviations the observed value is removed from the expected value. A bond length with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the average root-mean-square of all Z scores of the bond lengths.

Mol	Type	Chain	Res	Link	Bond lengths		
					Counts	RMSZ	#Z>2
1	1PA	A	48	1	10,15,16	0.91±0.06	0±0 (0±0%)

In the following table, the Counts columns list the number of angles for which Mogul statistics could be retrieved, the number of angles that are observed in the model and the number of angles that are defined in the chemical component dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond angle is the number of standard deviations the observed value is removed from the expected value. A bond angle with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the average root-mean-square of all Z scores of the bond angles.

Mol	Type	Chain	Res	Link	Bond angles		
					Counts	RMSZ	#Z>2
1	1PA	A	48	1	14,19,21	0.84±0.13	0±0 (0±0%)

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the chemical component dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
1	1PA	A	48	1	-	0±0,6,10,12	0±0,1,1,1

There are no bond-length outliers.

There are no bond-angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

6.5 Carbohydrates [i](#)

There are no carbohydrates in this entry.

6.6 Ligand geometry [i](#)

1 ligand is modelled in this entry.

In the following table, the Counts columns list the number of bonds for which Mogul statistics could be retrieved, the number of bonds that are observed in the model and the number of bonds that are defined in the chemical component dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond length is the number of standard deviations the observed value is removed from the expected value. A bond length with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the average root-mean-square of all Z scores of the bond lengths.

Mol	Type	Chain	Res	Link	Bond lengths		
					Counts	RMSZ	#Z>2
2	MH0	A	201	1	24,50,50	0.34±0.03	0±0 (0±0%)

In the following table, the Counts columns list the number of angles for which Mogul statistics could be retrieved, the number of angles that are observed in the model and the number of angles that are defined in the chemical component dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond angle is the number of standard deviations the observed value is removed from the expected value. A bond angle with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the average root-mean-square of all Z scores of the bond angles.

Mol	Type	Chain	Res	Link	Bond angles		
					Counts	RMSZ	#Z>2
2	MH0	A	201	1	17,82,82	0.94±0.10	0±0 (0±0%)

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the chemical component dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
2	MH0	A	201	1	-	0±0,10,54,54	0±0,0,8,8

There are no bond-length outliers.

There are no bond-angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

6.7 Other polymers ⓘ

There are no such molecules in this entry.

6.8 Polymer linkage issues ⓘ

There are no chain breaks in this entry.

7 Chemical shift validation

The completeness of assignment taking into account all chemical shift lists is 88% for the well-defined parts and 87% for the entire structure.

7.1 Chemical shift list 1

File name: 2n3y_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	1263
Number of shifts mapped to atoms	1242
Number of unparsed shifts	2
Number of shifts with mapping errors	19
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	23

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

- Entity instance (chain) must be specified. All 2 occurrences are reported below.

Shift ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
537	?	48	CMF	QD	7.206	0.001	1
538	?	48	CMF	QE	7.165	0.001	1

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

- Chain not found in structure. First 5 (of 19) occurrences are reported below.

Chain	Res	Type	Atom	Shift Data		
				Value	Uncertainty	Ambiguity
B	105	HEM	HB63	3.68	0.001	1
B	105	HEM	HB73	2.346	0.001	1
B	105	HEM	QM5	3.342	0.001	1
B	105	HEM	HA72	3.311	0.001	1
B	105	HEM	QM3	3.735	0.001	1

7.1.2 Chemical shift referencing ⓘ

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction \pm precision, ppm	Suggested action
$^{13}\text{C}_\alpha$	102	0.21 ± 0.20	None needed (< 0.5 ppm)
$^{13}\text{C}_\beta$	89	0.34 ± 0.14	None needed (< 0.5 ppm)
$^{13}\text{C}'$	100	0.26 ± 0.16	None needed (< 0.5 ppm)
^{15}N	88	-0.03 ± 0.27	None needed (< 0.5 ppm)

7.1.3 Completeness of resonance assignments ⓘ

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 88%, i.e. 981 atoms were assigned a chemical shift out of a possible 1111. 9 out of 9 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^1H	^{13}C	^{15}N
Backbone	417/429 (97%)	166/171 (97%)	173/174 (99%)	78/84 (93%)
Sidechain	525/598 (88%)	330/354 (93%)	191/218 (88%)	4/26 (15%)
Aromatic	39/84 (46%)	38/45 (84%)	0/35 (0%)	1/4 (25%)
Overall	981/1111 (88%)	534/570 (94%)	364/427 (85%)	83/114 (73%)

7.1.4 Statistically unusual chemical shifts ⓘ

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	80	MET	HG3	-3.75	4.30 – 0.50	-16.2
1	A	18	HIS	HD2	0.01	9.28 – 4.78	-15.6
1	A	18	HIS	HE1	0.38	10.53 – 5.43	-14.9
1	A	80	MET	HB3	-2.81	3.70 – 0.30	-14.2
1	A	80	MET	HG2	-2.05	4.23 – 0.63	-12.4
1	A	80	MET	HE2	-3.43	4.28 – -0.52	-11.1
1	A	80	MET	HE3	-3.43	4.28 – -0.52	-11.1
1	A	80	MET	HE1	-3.43	4.28 – -0.52	-11.1
1	A	29	GLY	HA2	-0.07	5.87 – 2.07	-10.6
1	A	80	MET	HB2	-0.26	3.73 – 0.33	-6.7
1	A	82	PHE	HB3	0.42	4.85 – 1.05	-6.6
1	A	18	HIS	HB3	0.66	5.00 – 1.10	-6.1
1	A	18	HIS	HB2	1.00	4.91 – 1.31	-5.9
1	A	32	LEU	HD11	-0.87	2.16 – -0.64	-5.8

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Mol	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	A	32	LEU	HD12	-0.87	2.16 – -0.64	-5.8
1	A	32	LEU	HD13	-0.87	2.16 – -0.64	-5.8
1	A	71	PRO	HG3	-0.01	3.56 – 0.26	-5.8
1	A	30	PRO	HD3	1.52	5.52 – 1.72	-5.5
1	A	97	TYR	HE2	5.46	7.86 – 5.56	-5.4
1	A	32	LEU	HD23	-0.76	2.14 – -0.66	-5.4
1	A	32	LEU	HD22	-0.76	2.14 – -0.66	-5.4
1	A	32	LEU	HD21	-0.76	2.14 – -0.66	-5.4
1	A	17	CYS	HB3	0.43	5.25 – 0.55	-5.3

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.

Random coil index (RCI) for chain A:

