

Full wwPDB NMR Structure Validation Report (i)

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PDB ID	:	7MLL
BMRB ID	:	50886
Title	:	Solution structure of Exenatide (exendin-4) in 30-vol% trifluoroethanol using
		CS-Rosetta
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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 types of validation reports are described at http://www.wwpdb.org/validation/2017/FAQs#types.

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)
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 85%.

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		Percentile Ranks	Va	lue
Clashscore			0	
Ramachandran outliers			0	
Sidechain outliers			0	
	Worse		Better	
	Percentile relativ	ve to all structures		
	Percentile relativ	ve to all NMR structures		
		Whole archive	NMR archive	P

Metric	Whole archive (#Entries)	NMR archive $(\#\operatorname{Entries})$
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 cha	in
1	А	40	62%	38%



2 Ensemble composition and analysis (i)

This entry contains 5 models. Model 1 is the overall representative, medoid model (most similar to other models).

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:10-A:34 (25)	0.83	1		

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 1 clusters. No single-model clusters were found.

Cluster number	Models		
1	1, 2, 3, 4, 5		



3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 575 atoms, of which 280 are hydrogens and 0 are deuteriums.

• Molecule 1 is a protein called Exendin-4.

Mol	Chain	Residues		A	Atom	5			Trace
1	٨	40	Total	С	Η	Ν	0	S	1
	A	40	575	184	280	50	60	1	1

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	40	NH2	-	amidation	UNP P26349



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: Exendin-4

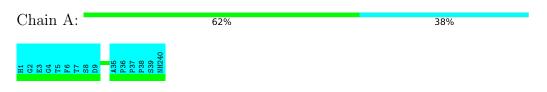
Chain A:	62%	38%
H1 62 75 75 77 738 09	A35 P37 P38 S39 NH240	

4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

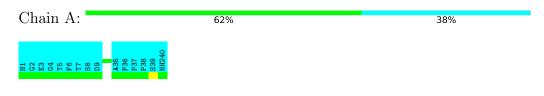
4.2.1 Score per residue for model 1 (medoid)

• Molecule 1: Exendin-4



4.2.2 Score per residue for model 2

• Molecule 1: Exendin-4





4.2.3 Score per residue for model 3

• Molecule 1: Exendin-4

Chain A:	62%	38%
H 22 23 24 23 25 H 28 23 28 28 28 28 28 28 29 28 28 29 28 29 29 29 29 29 29 29 29 29 29 29 29 29	A35 P36 P37 S39 NH240	

4.2.4 Score per residue for model 4

• Molecule 1: Exendin-4

Chain A:	62%	38%
H1 622 756 777 768 777 768 777	735 P37 P37 S39 NH240	

4.2.5 Score per residue for model 5

• Molecule 1: Exendin-4

Chain A:	62%	38%
H1 G2 F5 F5 F5 F5 F5 F33 A35 A35	P36 P37 S39 NH240	



5 Refinement protocol and experimental data overview (i)

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

Of the 3000 calculated structures, 5 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
CS-ROSETTA	structure calculation	
Rosetta	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)	working_cs.cif
Number of chemical shift lists	1
Total number of shifts	419
Number of shifts mapped to atoms	419
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	85%



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: NH2

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
All	All	980	970	970	-

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 (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	А	25/40~(62%)	25±0 (100±0%)	0±0 (0±0%)	0±0 (0±0%)	100 100
All	All	125/200~(62%)	125 (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	Perce	entiles
1	А	21/32~(66%)	21±0 (100±0%)	0±0 (0±0%)	100	100
All	All	105/160~(66%)	105 (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 (i)

The completeness of assignment taking into account all chemical shift lists is 85% for the well-defined parts and 86% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: assigned_chemical_shifts_1

7.1.1 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	419
Number of shifts mapped to atoms	419
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	1

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	${\rm Correction}\pm{\rm precision},ppm$	Suggested action
$^{13}C_{\alpha}$	39	-0.56 ± 0.25	Should be checked
$^{13}C_{\beta}$	34	0.34 ± 0.09	None needed (< 0.5 ppm)
$^{13}C'$	20		None (insufficient data)
¹⁵ N	33	0.16 ± 0.41	None needed (< 0.5 ppm)

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 85%, i.e. 286 atoms were assigned a chemical shift out of a possible 337. 0 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathrm{H}$	$^{13}\mathrm{C}$	$^{15}\mathbf{N}$
Backbone	108/126~(86%)	52/52~(100%)	32/50~(64%)	24/24~(100%)
Sidechain	160/189~(85%)	105/122~(86%)	52/60~(87%)	3/7~(43%)

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	Total	$^{1}\mathrm{H}$	$^{13}\mathrm{C}$	$^{15}\mathbf{N}$
Aromatic	18/22~(82%)	9/11~(82%)	8/10 (80%)	1/1~(100%)
Overall	286/337~(85%)	166/185~(90%)	92/120~(77%)	28/32~(88%)

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The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 86%, i.e. 416 atoms were assigned a chemical shift out of a possible 486. 0 out of 4 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathrm{H}$	$^{13}\mathrm{C}$	$^{15}\mathbf{N}$
Backbone	168/192~(88%)	76/79~(96%)	59/78~(76%)	33/35~(94%)
Sidechain	220/255~(86%)	143/165~(87%)	74/83~(89%)	3/7~(43%)
Aromatic	28/39~(72%)	14/20~(70%)	13/17~(76%)	1/2~(50%)
Overall	416/486~(86%)	233/264~(88%)	146/178~(82%)	37/44 (84%)

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.

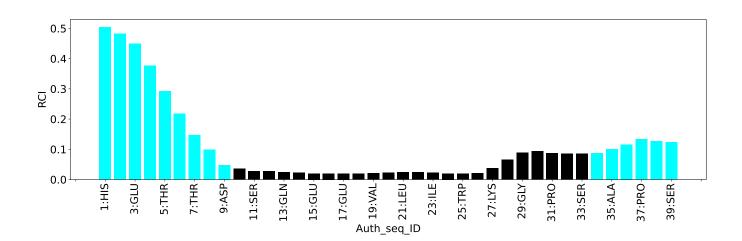
List Id	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	А	30	GLY	HA3	1.63	2.08 - 5.71	-6.2

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:







8 NMR restraints analysis (i)

No restraints data found



9 Distance violation analysis (i)

No distance restraints data found



10 Dihedral-angle violation analysis (i)

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

