

Full wwPDB NMR Structure Validation Report (i)

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PDB ID	:	3ZOB
Title	:	Solution structure of chicken Engrailed 2 homeodomain
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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 following versions of software and data (see references (1)) were used in the production of this report:

Cyrange	:	Kirchner and Güntert (2011)
$\operatorname{NmrClust}$:	Kelley et al. (1996)
$\operatorname{MolProbity}$:	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)
${ m ShiftChecker}$:	2.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.11

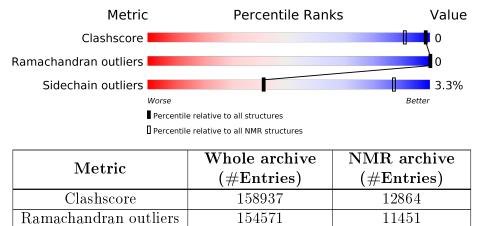
Sidechain outliers

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 95%.

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.



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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%

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Mol	Chain	Length	Quality of chain	
1	А	67	67%	33%



2 Ensemble composition and analysis (i)

This entry contains 20 models. Model 15 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative.

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:13-A:57 (45)	0.37	15	

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 3 single-model clusters were found.

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



3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 1125 atoms, of which 572 are hydrogens and 0 are deuteriums.

• Molecule 1 is a protein called HOMEOBOX PROTEIN ENGRAILED-2.

Mol	Chain	Residues			Atom	ıs			Trace
1	Λ	67	Total	С	Η	Ν	Ο	S	0
	A	67	1125	343	572	108	101	1	0

There are 7 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1	GLY	-	expression tag	UNP Q05917
A	2	PRO	-	expression tag	UNP Q05917
A	3	MET	-	expression tag	UNP Q05917
A	4	ALA	-	expression tag	UNP Q05917
A	5	SER	-	expression tag	UNP Q05917
A	66	GLN	-	expression tag	UNP Q05917
A	67	ALA	-	expression tag	UNP Q05917



4 Residue-property plots (i)

4.1 Average score per residue in the NMR ensemble

These plots are provided for all protein, RNA and DNA 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: HOMEOBOX PROTEIN ENGRAILED-2

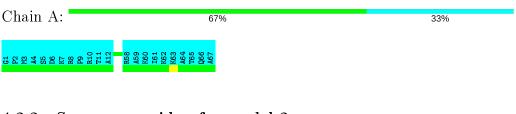


4.2 Scores per residue for each member of the ensemble

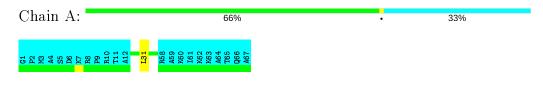
Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

• Molecule 1: HOMEOBOX PROTEIN ENGRAILED-2

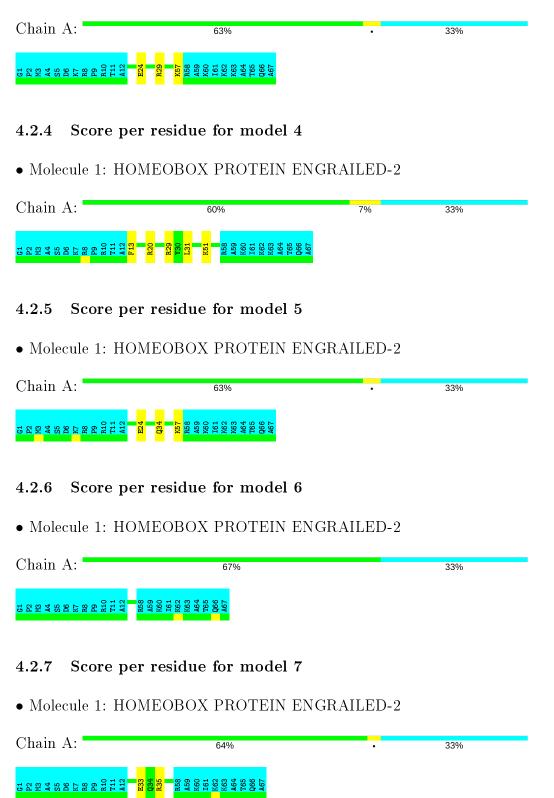


4.2.2 Score per residue for model 2





4.2.3 Score per residue for model 3



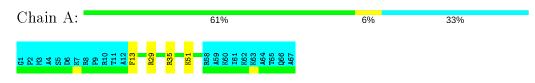


4.2.8 Score per residue for model 8

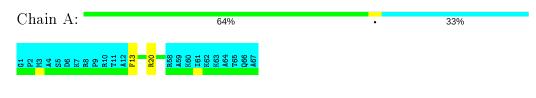
• Molecule 1: HOMEOBOX PROTEIN ENGRAILED-2

Chain A:	67%		33%
01 10 10 10 10 10 10 10 10 10 10 10 10 1	966 A67		
4.2.9 Score per residue	for model 9		
• Molecule 1: HOMEOBOX	PROTEIN ENGRAIL	ED-2	
Chain A:	64%	•	33%
61 71 71 71 71 71 71 71 71 71 71 71 71 71	K63 A64 Q66 A67		
4.2.10 Score per residu	e for model 10		
• Molecule 1: HOMEOBOX	PROTEIN ENGRAIL	ED-2	
Chain A:	64%	•	33%
61 85 85 85 85 85 85 85 86 86 86 86 86 86 86 86 86 86 86 86 86	K63 A64 165 A67 A67		
4.2.11 Score per residu	e for model 11		

• Molecule 1: HOMEOBOX PROTEIN ENGRAILED-2



4.2.12 Score per residue for model 12





4.2.13 Score per residue for model 13

Chain A:	66% .	33%
G1 P2 A3 A4 A5 A5 A12 A12 A12 A12 A58 A12 A12 A12 A12 A12 A12 A12 A12 A12 A12	1465 166 167	
4.2.14 Score per residue	for model 14	
• Molecule 1: HOMEOBOX	PROTEIN ENGRAILED-2	
Chain A:	64% ·	33%
G1 P2 M3 M3 M3 M3 M3 M3 M3 M3 M3 M3 M3 M3 M3	A64 165 165 166 166 166	
4.2.15 Score per residue	for model 15 (medoid)	
• Molecule 1: HOMEOBOX	PROTEIN ENGRAILED-2	
Chain A:	64% •	33%
61 72 75 75 75 75 75 75 75 75 75 75 75 75 75	X602 A664 1655 A667 A667	
4.2.16 Score per residue	for model 16	
• Molecule 1: HOMEOBOX	PROTEIN ENGRAILED-2	
Chain A:	66% .	33%
61 85 85 85 85 85 11 11 12 12 15 15 15 15 15 15 15 15 15 15 15 15 15	1969 1969 1969 1969 1969 1969 1969 1969	
4.2.17 Score per residue	for model 17	
• Molecule 1: HOMEOBOX	PROTEIN ENGRAILED-2	
Chain A:	66% .	33%
61 P2 A4 A55 A55 C111 T111 T111 T111 T111 T111 T111 T1	400 400 400 400 400 400 400 400 400 400	



4.2.18 Score per residue for model 18

Chain A:	64%		33%
61 72 72 75 75 71 711 711 711 711 711 711 711 71	161 161 165 166 165 165 165		
4.2.19 Score per resi	due for model 19		
• Molecule 1: HOMEOB	OX PROTEIN ENGRA	AILED-2	
Chain A:	64%	·	33%
61 85 85 85 85 85 85 85 85 85 85 85 85 85	1600 K62 A664 A664 A67 A67		
4.2.20 Score per resi	due for model 20		
• Molecule 1: HOMEOB	OX PROTEIN ENGRA	AILED-2	
Chain A:	64%	·	33%
61 10 11 11 11 11 11 11 11 11 11 11 11 11	460 161 161 165 165 165 165 165 165		



5 Refinement protocol and experimental data overview (i)

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

Of the 100 calculated structures, 20 were deposited, based on the following criterion: LOWEST ENERGY.

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

Software name	Classification	Version
XPLOR-NIH	refinement	
DYANA	structure solution	

The following table shows chemical shift validation statistics as aggregates over all chemical shift files. Detailed validation can be found in section 6 of this report.

Chemical shift file(s)	input_cs.cif
Number of chemical shift lists	1
Total number of shifts	924
Number of shifts mapped to atoms	924
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	95%

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

COVALENT-GEOMETRY INFOmissingINFO

5.1 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	А	387	386	386	0 ± 0
All	All	7740	7720	7720	1

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	$\mathrm{Clash}(\mathrm{\AA})$	Distance(Å)	Models	
Atom-1	Atom-2		Distance(A)	Worst	Total
1:A:31:LEU:H	1:A:31:LEU:HD22	0.41	1.76	16	1

5.2 Torsion angles (i)

5.2.1 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	ntiles
1	А	45/67~(67%)	$42 \pm 1 \ (93 \pm 2\%)$	$3\pm1~(7\pm2\%)$	0±0 (0±0%)	100	100
All	All	900/1340~(67%)	837 (93%)	63 (7%)	0 (0%)	100	100

There are no Ramachandran outliers.

5.2.2 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	Percer	ntiles
1	А	41/57~(72%)	40 ± 1 (97±3%)	$1 \pm 1 (3 \pm 3\%)$	41	87
All	All	820/1140 (72%)	793~(97%)	27 (3%)	41	87

All 9 unique residues with a non-rotameric sidechain are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	\mathbf{Res}	Type	Models (Total)
1	А	31	LEU	5
1	А	29	ARG	4
1	А	57	LYS	4
1	А	20	ARG	3
1	А	34	GLN	3
1	А	35	ARG	3
1	А	24	GLU	2
1	А	51	LYS	2

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Mol	Chain	Res	Type	Models (Total)
1	А	33	GLU	1

5.2.3 RNA (i)

There are no RNA molecules in this entry.

5.3 Non-standard residues in protein, DNA, RNA chains (i)

There are no non-standard protein/DNA/RNA residues in this entry.

5.4 Carbohydrates (i)

There are no carbohydrates in this entry.

5.5 Ligand geometry (i)

There are no ligands in this entry.

5.6 Other polymers (i)

There are no such molecules in this entry.

5.7 Polymer linkage issues (i)

There are no chain breaks in this entry.



6 Chemical shift validation (i)

The completeness of assignment taking into account all chemical shift lists is 95% for the well-defined parts and 93% for the entire structure.

6.1 Chemical shift list 1

File name: input_cs.cif

Chemical shift list name: En2HD_1.bmrb.csh

6.1.1 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	924
Number of shifts mapped to atoms	924
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	5

6.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	${\bf Correction}\pm{\bf precision},ppm$	Suggested action
$^{13}C_{\alpha}$	66	-0.26 ± 0.12	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	64	0.15 ± 0.13	None needed (< 0.5 ppm)
$^{13}C'$	66	-0.44 ± 0.14	None needed (< 0.5 ppm)
¹⁵ N	64	-0.16 ± 0.18	None needed (< 0.5 ppm)

6.1.3 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 95%, i.e. 598 atoms were assigned a chemical shift out of a possible 630. 0 out of 6 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathrm{H}$	$^{13}\mathrm{C}$	$^{15}\mathbf{N}$
Backbone	225/225~(100%)	90/90~(100%)	90/90~(100%)	45/45~(100%)
Sidechain	326/358~(91%)	192/211~(91%)	118/121~(98%)	16/26~(62%)

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0 1 1 0		
Continued from	previous	page
	1	1 0

	Total	$^{1}\mathbf{H}$	$^{13}\mathrm{C}$	$^{15}\mathbf{N}$
Aromatic	47/47~(100%)	25/25~(100%)	21/21~(100%)	1/1~(100%)
Overall	598/630~(95%)	307/326~(94%)	229/232~(99%)	62/72~(86%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 93%, i.e. 846 atoms were assigned a chemical shift out of a possible 912. 0 out of 6 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathrm{H}$	$^{13}\mathrm{C}$	15 N
Backbone	326/331~(98%)	130/132~(98%)	132/134~(99%)	64/65~(98%)
Sidechain	473/534 (89%)	282/317~(89%)	171/177~(97%)	20/40~(50%)
Aromatic	47/47~(100%)	25/25~(100%)	21/21~(100%)	1/1~(100%)
Overall	846/912~(93%)	437/474~(92%)	324/332~(98%)	85/106~(80%)

6.1.4 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.

Mol	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	А	57	LYS	HG2	-0.24	2.67 - 0.07	-6.2
1	А	57	LYS	HG3	-0.37	2.760.04	-6.2
1	А	21	LEU	HB3	-0.37	3.340.26	-5.3
1	А	57	LYS	HD3	0.38	2.75 - 0.45	-5.3
1	А	57	LYS	HB3	0.37	3.10 - 0.40	-5.1

6.1.5 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.

Random coil index (RCI) for chain A:



