wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	2LSP
Title	:	solution structures of BRD4 second bromodomain with NF-kB-K310ac peptide
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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 (i) symbol.

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

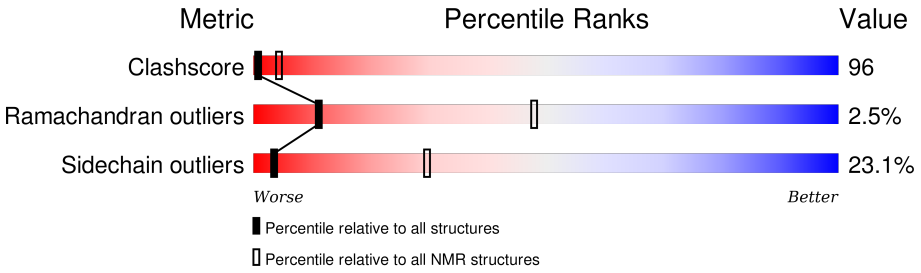
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	1.7.1 (RC1), CSD as537be (2016)
Percentile statistics	:	20151230.v01 (using entries in the PDB archive December 30th 2015)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	rb-20027457
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	rb-20027457

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 82%.

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 <=5%

Mol	Chain	Length	Quality of chain
1	A	13	100%
2	B	128	16% 45% 18% 20%

2 Ensemble composition and analysis

This entry contains 20 models. Model 17 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *lowest energy*.

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	B:353-B:454 (102)	0.10	17

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 4 single-model clusters were found.

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

3 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 2294 atoms, of which 1141 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called NF-kB-K310ac peptide.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	13	239	74	123	19	22	1	0

- Molecule 2 is a protein called Bromodomain-containing protein 4.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
2	B	128	2055	658	1018	175	193	11	0

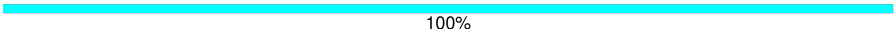
4 Residue-property plots

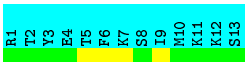
4.1 Average score per residue in the NMR ensemble

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: NF-kB-K310ac peptide

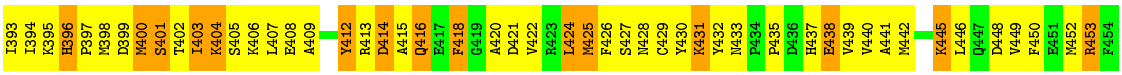
Chain A:

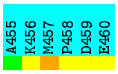




- Molecule 2: Bromodomain-containing protein 4

Chain B:



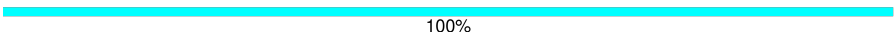


4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 17. Colouring as in section 4.1 above.

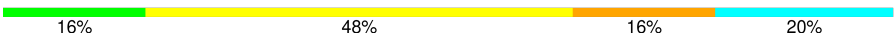
- Molecule 1: NF-kB-K310ac peptide

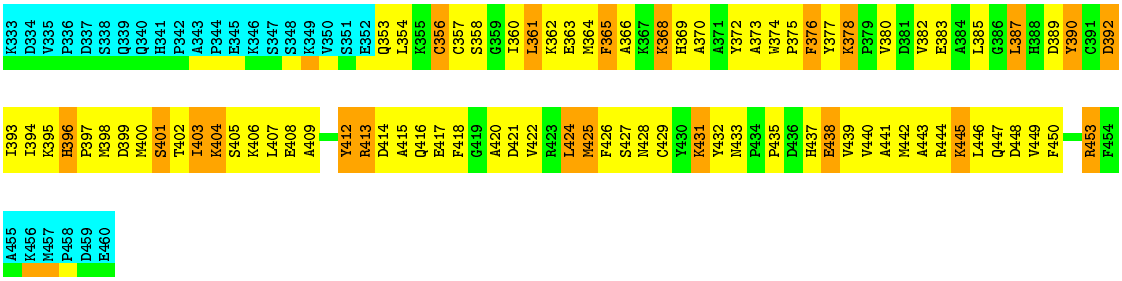
Chain A:



- Molecule 2: Bromodomain-containing protein 4

Chain B:




K333 D334 V335 P336 D337 S338 Q339 Q340 H341 P342 A343 P344 E345 K346 S347 S348 K349 V350 S351 E352 Q353 L354 K355 C356 C357 S358 G359 I360 L361 K362 E363 M364 F365 A366 K367 K368 H369 A370 A371 Y372 A373 W374 P375 F376 Y377 K378 P379 V380 D381 V382 E383 A384 L385 G386 L387 H388 D389 Y390 C391 D392
I393 I394 K395 H396 P397 M398 D399 M400 S401 T402 I403 K404 S405 K406 L407 E408 A409 Y412 R413 D414 A415 Q416 E417 F418 G419 A420 D421 V422 R423 L424 M425 F426 S427 N428 C429 Y430 K431 Y432 N433 P434 P435 D436 H437 E438 V439 V440 A441 M442 A443 R444 K445 L446 Q447 D448 V449 F450 R453 F454
A455 K456 M457 P458 D459 E460

5 Refinement protocol and experimental data overview

The models were refined using the following method: *DGSA-distance geometry simulated annealing, torsion angle dynamics.*

Of the 200 calculated structures, 20 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
ARIA	refinement	2.3
TALOS	geometry optimization	3.70F1
CNS	structure solution	1.21
CNS	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)	2lsp_cs.str
Number of chemical shift lists	1
Total number of shifts	1435
Number of shifts mapped to atoms	1435
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	82%

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

6 Model quality

6.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: ALY

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
2	B	0.85±0.01	0±0/860 (0.0±0.0%)	0.93±0.01	3±0/1157 (0.2±0.0%)
All	All	0.85	0/17200 (0.0%)	0.93	56/23140 (0.2%)

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(^{o})	Ideal(^{o})	Models	
								Worst	Total
2	B	412	TYR	CB-CG-CD2	7.52	125.51	121.00	13	20
2	B	412	TYR	CB-CG-CD1	-7.17	116.69	121.00	13	20
2	B	418	PHE	CB-CG-CD1	-5.86	116.70	120.80	5	16

There are no chirality outliers.

There are no planarity outliers.

6.2 Too-close contacts

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	0	0	0	0±0
2	B	837	825	821	159±6
All	All	16740	16500	16420	3187

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 96.

5 of 338 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
2:B:373:ALA:HB2	2:B:446:LEU:HD21	1.11	1.22	16	20
2:B:372:TYR:HA	2:B:442:MET:SD	0.92	2.03	17	20
2:B:372:TYR:CG	2:B:445:LYS:HD2	0.92	1.98	8	7
2:B:372:TYR:CD2	2:B:445:LYS:HG2	0.91	2.00	12	4
2:B:357:CYS:HB3	2:B:418:PHE:CD1	0.91	2.01	5	20

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	0	-	-	-	-
2	B	102/128 (80%)	85±1 (83±1%)	15±1 (15±1%)	3±1 (3±1%)	11 48
All	All	2040/2820 (72%)	1691 (83%)	297 (15%)	52 (3%)	11 48

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

Mol	Chain	Res	Type	Models (Total)
2	B	387	LEU	20
2	B	390	TYR	12
2	B	414	ASP	12
2	B	373	ALA	7
2	B	367	LYS	1

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	0	-	-	-
2	B	89/113 (79%)	68±2 (77±2%)	21±2 (23±2%)	3 30
All	All	1780/2500 (71%)	1368 (77%)	412 (23%)	3 30

5 of 37 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)
2	B	403	ILE	20
2	B	368	LYS	20
2	B	361	LEU	20
2	B	365	PHE	20
2	B	392	ASP	20

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	ALY	A	7	1	9,11,12	0.58±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	ALY	A	7	1	10,12,14	1.11±0.05	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	ALY	A	7	1	-	0±0,8,10,12	0±0,0,0,0

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)

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 82% for the well-defined parts and 78% for the entire structure.

7.1 Chemical shift list 1

File name: 2lsp_cs.str

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	1435
Number of shifts mapped to atoms	1435
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	19

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	127	-0.46 ± 0.20	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	123	0.33 ± 0.14	None needed (< 0.5 ppm)
$^{13}C'$	10	0.00 ± 0.00	None needed (< 0.5 ppm)
^{15}N	114	0.42 ± 0.31	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 82%, i.e. 1084 atoms were assigned a chemical shift out of a possible 1318. 13 out of 13 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	397/500 (79%)	192/199 (96%)	110/204 (54%)	95/97 (98%)
Sidechain	582/672 (87%)	367/400 (92%)	213/242 (88%)	2/30 (7%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	105/146 (72%)	68/76 (89%)	36/61 (59%)	1/9 (11%)
Overall	1084/1318 (82%)	627/675 (93%)	359/507 (71%)	98/136 (72%)

7.1.4 Statistically unusual chemical shifts (i)

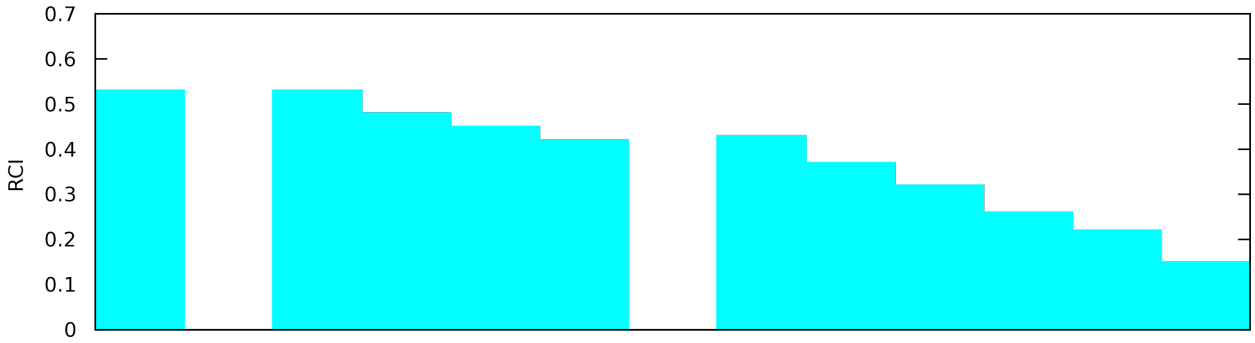
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

Mol	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
2	B	347	SER	C	8.45	183.48 – 165.88	-94.4
2	B	386	GLY	C	7.90	183.33 – 164.53	-88.3
2	B	346	LYS	C	8.51	186.58 – 166.78	-84.9
2	B	385	LEU	C	7.48	187.06 – 166.96	-84.3
2	B	430	TYR	C	7.61	185.42 – 165.42	-83.9
2	B	447	GLN	C	11.57	186.20 – 166.50	-83.6
2	B	388	HIS	C	8.30	185.27 – 165.27	-83.5
2	B	437	HIS	C	9.41	185.27 – 165.27	-82.9
2	B	384	ALA	C	7.86	188.57 – 166.97	-78.7
2	B	366	ALA	C	9.11	188.57 – 166.97	-78.1
2	B	375	PRO	HG2	-0.72	3.48 – 0.38	-8.6
2	B	447	GLN	HB2	0.07	3.30 – 0.80	-7.9
2	B	375	PRO	HB2	-0.35	3.82 – 0.32	-6.9
2	B	357	CYS	HB2	0.35	5.20 – 0.70	-5.8
2	B	422	VAL	HG11	-0.67	2.13 – -0.47	-5.8
2	B	422	VAL	HG13	-0.67	2.13 – -0.47	-5.8
2	B	422	VAL	HG12	-0.67	2.13 – -0.47	-5.8
2	B	375	PRO	HD2	1.62	5.45 – 1.85	-5.6
2	B	439	VAL	H	11.70	11.69 – 4.89	5.0

7.1.5 Random Coil Index (RCI) plots (i)

The images below report *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:



Random coil index (RCI) for chain B: