

## wwPDB EM Validation Summary Report (i)

#### Feb 25, 2024 – 11:51 AM EST

PDB ID	:	6X50
EMDB ID	:	EMD-22045
Title	:	Mfd-bound E.coli RNA polymerase elongation complex - V state
Authors	:	Llewelyn, E.; Chen, J.; Kang, J.Y.; Darst, S.A.
Deposited on	:	2020-05-24
Resolution	:	3.30 Å(reported)

This is a wwPDB EM Validation Summary 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/EMValidationReportHelp 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:

EMDB validation analysis	:	0.0.1. dev 70
Mogul	:	1.8.5 (274361), CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7(2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.13
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36

## 1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:  $ELECTRON\ MICROSCOPY$ 

The reported resolution of this entry is 3.30 Å.

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	$egin{array}{c} { m Whole \ archive} \ (\#{ m Entries}) \end{array}$	${f EM} {f structures} \ (\#{f Entries})$
Clashscore	158937	4297
Ramachandran outliers	154571	4023
Sidechain outliers	154315	3826
RNA backbone	4643	859

The table below summarises the geometric issues observed across the polymeric chains and their fit to the map. The red, orange, yellow and green segments of the bar indicate the fraction of residues that contain outliers for  $\geq=3, 2, 1$  and 0 types of geometric quality criteria respectively. 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  $\leq=5\%$  The upper red bar (where present) indicates the fraction of residues that have poor fit to the EM map (all-atom inclusion < 40%). The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain	
1	А	1148	<b>•</b> 80%	19% •
2	G	329	52% 16%	32%
2	Н	329	50% 16% ·	34%
3	Ι	1342	- 78%	20% •
4	J	1407	5%	18% 5%
5	K	91	73%	14% 13%
6	R	20	30% 10% 5% 55%	

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Mol	Chain	Length	Quality of chain						
7	Р	64	56%	30%	14%				
8	Q	64	42%	33%	• 23%				



## 2 Entry composition (i)

There are 11 unique types of molecules in this entry. The entry contains 36077 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

• Molecule 1 is a protein called Transcription-repair-coupling factor.

Mol	Chain	Residues		А	AltConf	Trace			
1	А	1143	Total 8946	C 5661	N 1598	O 1651	S 36	0	0

• Molecule 2 is a protein called DNA-directed RNA polymerase subunit alpha.

Mol	Chain	Residues		Ate	AltConf	Trace			
2	G	224	Total 1725	C 1080	N 303	O 336	S 6	0	0
2	Н	217	Total 1667	C 1043	N 293	O 325	${f S}{f 6}$	0	0

• Molecule 3 is a protein called DNA-directed RNA polymerase subunit beta.

Mol	Chain	Residues		A	AltConf	Trace			
2	Т	1216	Total	С	Ν	Ο	S	0	0
3	1	1510	10368	6507	1808	2010	43	0	0

• Molecule 4 is a protein called DNA-directed RNA polymerase subunit beta'.

Mol	Chain	Residues		Α	AltConf	Trace			
4	J	1336	Total 10383	C 6524	N 1852	O 1957	${ m S}{ m 50}$	0	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
J	1384	VAL	MET	conflict	UNP A0A4S1NBU2

• Molecule 5 is a protein called DNA-directed RNA polymerase subunit omega.

Mol	Chain	Residues		At	oms	AltConf	Trace		
5	K	79	Total 627	C 382	N 118	O 126	S 1	0	0



• Molecule 6 is a RNA chain called RNA (20-mer).

Mol	Chain	Residues	Atoms					AltConf	Trace
6	R	9	Total 201	C 89	N 42	O 61	Р 9	0	0

• Molecule 7 is a DNA chain called DNA (64-MER).

Mol	Chain	Residues		A	toms		AltConf	Trace	
7	Р	55	Total 1112	C 531	N 192	O 335	Р 54	0	0

• Molecule 8 is a DNA chain called DNA (64-MER).

Mol	Chain	Residues		A	toms		AltConf	Trace	
8	Q	49	Total 1013	C 478	N 197	0 289	Р 49	0	0

• Molecule 9 is ADENOSINE-5'-TRIPHOSPHATE (three-letter code: ATP) (formula:  $C_{10}H_{16}N_5O_{13}P_3$ ) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues		AltConf				
0	Λ	1	Total	С	Ν	Ο	Р	0
9	А	1	31	10	5	13	3	0

• Molecule 10 is MAGNESIUM ION (three-letter code: MG) (formula: Mg).



Mol	Chain	Residues	Atoms	AltConf		
10	А	1	Total Mg 1 1	0		
10	J	1	Total Mg 1 1	0		

• Molecule 11 is ZINC ION (three-letter code: ZN) (formula: Zn).

Mol	Chain	Residues	Atoms	AltConf		
11	J	2	Total Zn 2 2	0		



## 3 Residue-property plots (i)

These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and atom inclusion in map density. Residues are color-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. A red diamond above a residue indicates a poor fit to the EM map for this residue (all-atom inclusion < 40%). Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

• Molecule 1: Transcription-repair-coupling factor



Chain G:

52%

32%



16%



#### 

• Molecule 2: DNA-directed RNA polymerase subunit alpha



#### • Molecule 3: DNA-directed RNA polymerase subunit beta

Cha	in	I:	•												7	78%	6															20	1%			•				
MET V2	R10	LII R12	K13 D14		K17 D18	OTU	D23	Y26		L32	<mark>036</mark>		1 <del>4</del> 1	E50	ae 1	Y62	<b>S63</b>	G64 N65	S66	E67 ree		Y73	R74 1.75		F80	E84	C85	486 187		L100	V103	110 <del>4</del> Y105	E106		0116	1117	G125	E126	L'IZ/	T1 <u>31</u>
T135	1138	T141	H150	R151	D168	5159 S159	D160	K161	H165		V170	1177	P178	R180		W183 1.184	D185	V101	TATA	L194	<b>P107</b>	ICTU	R200	R202	K203	L204	L221	<del>и ССШ</del>	E-220	F230	N235	K236		M239 F240			R245 L246	R247	uzto E249	1255
E256 A257	V263	TOGO	T270		R275	TORE		12 <mark>92</mark>	905		N314	L322		L336	N339	D340	L341 D342	H343	7947	101	T350	V364		P375	0/04	N387	S391	E392	D393	R407	R411		D427	K431		I435	I445	1453	R454	E461
R465	V469	E472	K476	E477	R478 1 4 7 9	5480	L481	L484		L487	A495	K496	G507		F514	R528	R529	1530	L538	T539	0540	A543	R548	D549	V550	P560	1561	E565	G566	P567 N568	1569	S576	V577		r591 R592	K693	V594	E602	S607	A608
I609 E610 E611		GION	A619	D624	1633		R637	S638 K639		L644 E645		V650	D654		T657	V660	V661	A665		R678	A689	V690	P691 T692	L693	R694	K697		1/02	<mark>V708</mark>	V714	T7 15		-	R720	1732	-	V736	E7 45	1748	





• Molecule 4: DNA-directed RNA polymerase subunit beta'







# 4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C1	Depositor
Number of particles used	103000	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE	Depositor
	CORRECTION	
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose $(e^-/\text{\AA}^2)$	60	Depositor
Minimum defocus (nm)	Not provided	
Maximum defocus (nm)	Not provided	
Magnification	Not provided	
Image detector	GATAN K2 SUMMIT (4k x 4k)	Depositor
Maximum map value	43.752	Depositor
Minimum map value	-24.685	Depositor
Average map value	0.004	Depositor
Map value standard deviation	1.079	Depositor
Recommended contour level	3.5	Depositor
Map size (Å)	390.0, 390.0, 390.0	wwPDB
Map dimensions	300, 300, 300	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.3, 1.3, 1.3	Depositor



## 5 Model quality (i)

### 5.1 Standard geometry (i)

Bond lengths and bond angles in the following residue types are not validated in this section: ATP, ZN, MG

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

Mal	Chain	Bond	lengths	B	ond angles
WIOI	Unain	RMSZ	# Z  > 5	RMSZ	# Z  > 5
1	А	0.34	0/9124	0.53	1/12371~(0.0%)
2	G	0.30	0/1745	0.54	2/2366~(0.1%)
2	Н	0.27	0/1686	0.57	2/2286~(0.1%)
3	Ι	0.31	0/10533	0.52	1/14214~(0.0%)
4	J	0.29	0/10540	0.53	3/14231~(0.0%)
5	K	0.27	0/629	0.45	0/847
6	R	0.45	0/226	0.92	0/352
7	Р	0.63	0/1242	0.97	0/1913
8	Q	0.59	0/1138	0.92	3/1753~(0.2%)
All	All	0.34	0/36863	0.57	12/50333~(0.0%)

There are no bond length outliers.

The worst 5 of 12 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
3	Ι	1151	LEU	CA-CB-CG	7.93	133.53	115.30
8	Q	41	DA	O4'-C4'-C3'	-6.80	101.78	104.50
8	Q	41	DA	C3'-C2'-C1'	-6.47	94.73	102.50
1	А	784	LEU	CB-CG-CD1	-6.46	100.01	111.00
2	Н	48	LEU	CA-CB-CG	6.06	129.24	115.30

There are no chirality outliers.

There are no planarity outliers.

### 5.2 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 the chain respectively. The H(added) column lists the number of hydrogen



Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	А	8946	0	8855	173	0
2	G	1725	0	1763	30	0
2	Н	1667	0	1699	41	0
3	Ι	10368	0	10379	201	0
4	J	10383	0	10597	164	0
5	K	627	0	634	12	0
6	R	201	0	98	2	0
7	Р	1112	0	622	19	0
8	Q	1013	0	549	15	0
9	А	31	0	12	1	0
10	А	1	0	0	0	0
10	J	1	0	0	0	0
11	J	2	0	0	0	0
All	All	36077	0	35208	609	0

atoms added and optimized by MolProbity. The Clashes column lists the number of clashes within the asymmetric unit, whereas Symm-Clashes lists symmetry-related clashes.

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 9.

The worst 5 of 609 close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
3:I:62:TYR:CZ	3:I:476:LYS:HD3	1.48	1.47
1:A:780:PRO:HG2	1:A:783:ARG:CG	1.50	1.39
1:A:604:ASP:CB	1:A:778:THR:CG2	2.07	1.32
1:A:604:ASP:CB	1:A:778:THR:HG21	1.61	1.30
3:I:62:TYR:CZ	3:I:476:LYS:CD	2.14	1.30

There are no symmetry-related clashes.

#### 5.3 Torsion angles (i)

#### 5.3.1 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 EM 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	1141/1148 (99%)	1030 (90%)	100 (9%)	11 (1%)	15	46
2	G	220/329~(67%)	199 (90%)	21 (10%)	0	100	100
2	Н	213/329~(65%)	195 (92%)	17 (8%)	1 (0%)	29	61
3	Ι	1312/1342 (98%)	1218 (93%)	94 (7%)	0	100	100
4	J	1330/1407~(94%)	1234 (93%)	96 (7%)	0	100	100
5	Κ	77/91~(85%)	71 (92%)	6 (8%)	0	100	100
All	All	4293/4646 (92%)	3947 (92%)	334 (8%)	12 (0%)	44	71

5 of 12 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	А	118	CYS
1	А	120	HIS
1	А	467	PRO
1	А	988	ALA
1	А	990	ARG

#### 5.3.2 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 EM 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	ntiles
1	А	928/973~(95%)	924 (100%)	4 (0%)	91	95
2	G	191/286~(67%)	191 (100%)	0	100	100
2	Н	184/286~(64%)	183 (100%)	1 (0%)	88	93
3	Ι	1132/1157~(98%)	1131 (100%)	1 (0%)	93	97
4	J	1118/1168~(96%)	1114 (100%)	4 (0%)	91	95
5	Κ	67/75~(89%)	67~(100%)	0	100	100
All	All	3620/3945~(92%)	3610 (100%)	10 (0%)	92	96

 $5~{\rm of}~10$  residues with a non-rotameric side chain are listed below:

Mol	Chain	Res	Type
4	J	207	GLU
	-	-	

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Mol	Chain	Res	Type
4	J	320	ASN
4	J	1373	ARG
1	А	952	ILE
2	Н	191	ARG

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 7 such sidechains are listed below:

Mol	Chain	Res	Type
3	Ι	120	GLN
3	Ι	799	ASN
4	J	865	HIS
4	J	424	ASN
1	А	821	ASN

#### 5.3.3 RNA (i)

Mol	Chain	Analysed	Backbone Outliers	Pucker Outliers
6	R	8/20~(40%)	1 (12%)	0

All (1) RNA backbone outliers are listed below:

Mol	Chain	Res	Type
6	R	14	А

There are no RNA pucker outliers to report.

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

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

#### 5.5 Carbohydrates (i)

There are no monosaccharides in this entry.

### 5.6 Ligand geometry (i)

Of 5 ligands modelled in this entry, 4 are monoatomic - leaving 1 for Mogul analysis.



In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or 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 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| > 2 is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mal	Tuno	Chain	Dog	Tink	Bo	ond leng	ths	B	ond ang	les
WIOI	Type	Ullalli	nes	LIIIK	Counts	RMSZ	# Z  > 2	Counts	RMSZ	# Z  > 2
9	ATP	А	2000	10	26,33,33	0.90	1 (3%)	31,52,52	1.61	6 (19%)

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
9	ATP	А	2000	10	-	3/18/38/38	0/3/3/3

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	$\mathrm{Ideal}(\mathrm{\AA})$
9	А	2000	ATP	C5-C4	2.27	1.46	1.40

The worst 5 of 6 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms		$Observed(^{o})$	$Ideal(^{o})$
9	А	2000	ATP	PA-O3A-PB	-3.99	119.12	132.83
9	А	2000	ATP	PB-O3B-PG	-3.47	120.94	132.83
9	А	2000	ATP	N3-C2-N1	-3.13	123.78	128.68
9	А	2000	ATP	C3'-C2'-C1'	2.87	105.31	100.98
9	А	2000	ATP	C4-C5-N7	-2.73	106.56	109.40

There are no chirality outliers.

All (3) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
9	А	2000	ATP	C5'-O5'-PA-O1A
9	А	2000	ATP	O4'-C4'-C5'-O5'
9	А	2000	ATP	C5'-O5'-PA-O3A

There are no ring outliers.



1	monomer	is	involved	in	1	short contact:	
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Mol	Chain	Res Type		Clashes	Symm-Clashes	
9	А	2000	ATP	1	0	

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less then 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.



### 5.7 Other polymers (i)

There are no such residues in this entry.



### 5.8 Polymer linkage issues (i)

There are no chain breaks in this entry.



## 6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-22045. These allow visual inspection of the internal detail of the map and identification of artifacts.

No raw map or half-maps were deposited for this entry and therefore no images, graphs, etc. pertaining to the raw map can be shown.

### 6.1 Orthogonal projections (i)

#### 6.1.1 Primary map



The images above show the map projected in three orthogonal directions.

### 6.2 Central slices (i)

#### 6.2.1 Primary map



X Index: 150

Y Index: 150





The images above show central slices of the map in three orthogonal directions.

### 6.3 Largest variance slices (i)

#### 6.3.1 Primary map



X Index: 152

Y Index: 149

Z Index: 169

The images above show the largest variance slices of the map in three orthogonal directions.

#### 6.4 Orthogonal standard-deviation projections (False-color) (i)

#### 6.4.1 Primary map



The images above show the map standard deviation projections with false color in three orthogonal directions. Minimum values are shown in green, max in blue, and dark to light orange shades represent small to large values respectively.



#### 6.5 Orthogonal surface views (i)

6.5.1 Primary map



The images above show the 3D surface view of the map at the recommended contour level 3.5. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

### 6.6 Mask visualisation (i)

This section was not generated. No masks/segmentation were deposited.



## 7 Map analysis (i)

This section contains the results of statistical analysis of the map.

### 7.1 Map-value distribution (i)



The map-value distribution is plotted in 128 intervals along the x-axis. The y-axis is logarithmic. A spike in this graph at zero usually indicates that the volume has been masked.



#### 7.2 Volume estimate (i)



The volume at the recommended contour level is  $304 \text{ nm}^3$ ; this corresponds to an approximate mass of 275 kDa.

The volume estimate graph shows how the enclosed volume varies with the contour level. The recommended contour level is shown as a vertical line and the intersection between the line and the curve gives the volume of the enclosed surface at the given level.



### 7.3 Rotationally averaged power spectrum (i)



\*Reported resolution corresponds to spatial frequency of 0.303  ${\rm \AA^{-1}}$ 



## 8 Fourier-Shell correlation (i)

This section was not generated. No FSC curve or half-maps provided.



## 9 Map-model fit (i)

This section contains information regarding the fit between EMDB map EMD-22045 and PDB model 6X50. Per-residue inclusion information can be found in section 3 on page 7.

### 9.1 Map-model overlay (i)



The images above show the 3D surface view of the map at the recommended contour level 3.5 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.



### 9.2 Q-score mapped to coordinate model (i)



The images above show the model with each residue coloured according its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions at which atoms can be resolved. Low Q-score values may therefore be expected for many entries.

#### 9.3 Atom inclusion mapped to coordinate model (i)



The images above show the model with each residue coloured according to its atom inclusion. This shows to what extent they are inside the map at the recommended contour level (3.5).



### 9.4 Atom inclusion (i)



At the recommended contour level, 90% of all backbone atoms, 85% of all non-hydrogen atoms, are inside the map.



#### Map-model fit summary (i) 9.5

The table lists the average atom inclusion at the recommended contour level (3.5) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score	1.0
All	0.8530	0.4940	
А	0.8930	0.4870	
G	0.8780	0.5340	
Н	0.8530	0.5060	
I	0.8470	0.5100	
J	0.8130	0.4940	
K	0.7680	0.4920	
Р	0.9270	0.4300	
Q	0.8710	0.3870	0.0 • <0.0
R	0.9450	0.5140	

