wwPDB EM Validation Summary Report ⓘ

May 4, 2024 – 12:23 pm BST

PDB ID	:	6I00
EMDB ID	:	EMD-0320
Title	:	Cryo-EM informed directed evolution of Nitrilase 4 leads to a change in quaternary structure.
Authors	:	Mulelu, A.E.; Woodward, J.D.
Deposited on	:	2018-10-24
Resolution	:	3.40 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 ⓘ 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 ⓘ) were used in the production of this report:

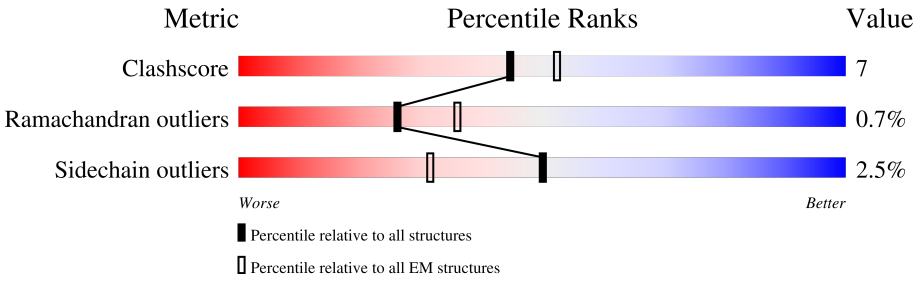
EMDB validation analysis	:	0.0.1.dev92
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	FAILED
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36.2

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 3.40 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	Whole archive (#Entries)	EM structures (#Entries)
Clashscore	158937	4297
Ramachandran outliers	154571	4023
Sidechain outliers	154315	3826

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 >=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 <=5%

Mol	Chain	Length	Quality of chain
1	A	361	69% 9% • 20%
1	B	361	66% 14% 20%
1	C	361	68% 11% • 20%
1	D	361	66% 14% • 20%
1	E	361	66% 12% • 20%
1	F	361	66% 13% 20%
1	G	361	66% 12% • 20%
1	H	361	66% 13% 20%
1	I	361	66% 12% • 20%

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Mol	Chain	Length	Quality of chain
1	J	361	
1	K	361	
1	L	361	

2 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 26712 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 Bifunctional nitrilase/nitrile hydratase NIT4.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	J	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	I	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	H	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	G	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	F	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	E	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	D	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	C	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	B	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	A	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	L	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		
1	K	289	Total	C	N	O	S	0	0
			2226	1425	381	407	13		

There are 72 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
J	356	HIS	-	expression tag	UNP P46011
J	357	HIS	-	expression tag	UNP P46011
J	358	HIS	-	expression tag	UNP P46011
J	359	HIS	-	expression tag	UNP P46011
J	360	HIS	-	expression tag	UNP P46011
J	361	HIS	-	expression tag	UNP P46011

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Chain	Residue	Modelled	Actual	Comment	Reference
I	356	HIS	-	expression tag	UNP P46011
I	357	HIS	-	expression tag	UNP P46011
I	358	HIS	-	expression tag	UNP P46011
I	359	HIS	-	expression tag	UNP P46011
I	360	HIS	-	expression tag	UNP P46011
I	361	HIS	-	expression tag	UNP P46011
H	356	HIS	-	expression tag	UNP P46011
H	357	HIS	-	expression tag	UNP P46011
H	358	HIS	-	expression tag	UNP P46011
H	359	HIS	-	expression tag	UNP P46011
H	360	HIS	-	expression tag	UNP P46011
H	361	HIS	-	expression tag	UNP P46011
G	356	HIS	-	expression tag	UNP P46011
G	357	HIS	-	expression tag	UNP P46011
G	358	HIS	-	expression tag	UNP P46011
G	359	HIS	-	expression tag	UNP P46011
G	360	HIS	-	expression tag	UNP P46011
G	361	HIS	-	expression tag	UNP P46011
F	356	HIS	-	expression tag	UNP P46011
F	357	HIS	-	expression tag	UNP P46011
F	358	HIS	-	expression tag	UNP P46011
F	359	HIS	-	expression tag	UNP P46011
F	360	HIS	-	expression tag	UNP P46011
F	361	HIS	-	expression tag	UNP P46011
E	356	HIS	-	expression tag	UNP P46011
E	357	HIS	-	expression tag	UNP P46011
E	358	HIS	-	expression tag	UNP P46011
E	359	HIS	-	expression tag	UNP P46011
E	360	HIS	-	expression tag	UNP P46011
E	361	HIS	-	expression tag	UNP P46011
D	356	HIS	-	expression tag	UNP P46011
D	357	HIS	-	expression tag	UNP P46011
D	358	HIS	-	expression tag	UNP P46011
D	359	HIS	-	expression tag	UNP P46011
D	360	HIS	-	expression tag	UNP P46011
D	361	HIS	-	expression tag	UNP P46011
C	356	HIS	-	expression tag	UNP P46011
C	357	HIS	-	expression tag	UNP P46011
C	358	HIS	-	expression tag	UNP P46011
C	359	HIS	-	expression tag	UNP P46011
C	360	HIS	-	expression tag	UNP P46011
C	361	HIS	-	expression tag	UNP P46011

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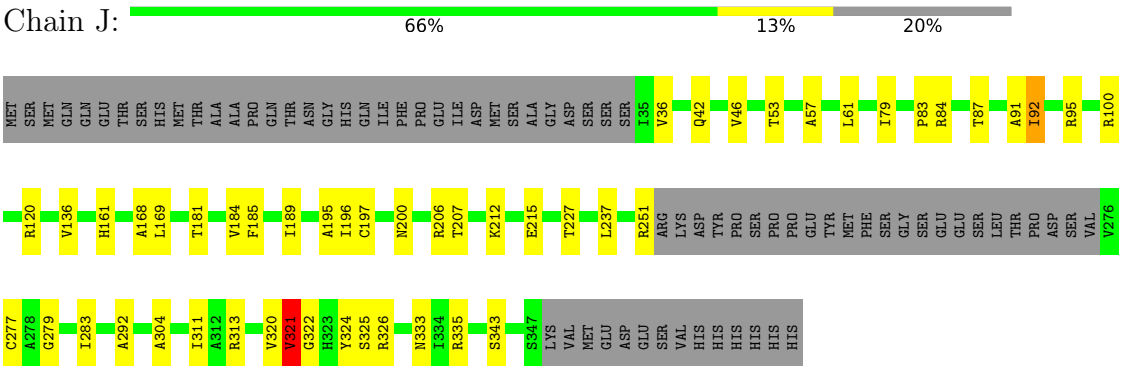
Chain	Residue	Modelled	Actual	Comment	Reference
B	356	HIS	-	expression tag	UNP P46011
B	357	HIS	-	expression tag	UNP P46011
B	358	HIS	-	expression tag	UNP P46011
B	359	HIS	-	expression tag	UNP P46011
B	360	HIS	-	expression tag	UNP P46011
B	361	HIS	-	expression tag	UNP P46011
A	356	HIS	-	expression tag	UNP P46011
A	357	HIS	-	expression tag	UNP P46011
A	358	HIS	-	expression tag	UNP P46011
A	359	HIS	-	expression tag	UNP P46011
A	360	HIS	-	expression tag	UNP P46011
A	361	HIS	-	expression tag	UNP P46011
L	356	HIS	-	expression tag	UNP P46011
L	357	HIS	-	expression tag	UNP P46011
L	358	HIS	-	expression tag	UNP P46011
L	359	HIS	-	expression tag	UNP P46011
L	360	HIS	-	expression tag	UNP P46011
L	361	HIS	-	expression tag	UNP P46011
K	356	HIS	-	expression tag	UNP P46011
K	357	HIS	-	expression tag	UNP P46011
K	358	HIS	-	expression tag	UNP P46011
K	359	HIS	-	expression tag	UNP P46011
K	360	HIS	-	expression tag	UNP P46011
K	361	HIS	-	expression tag	UNP P46011

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. 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. 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.

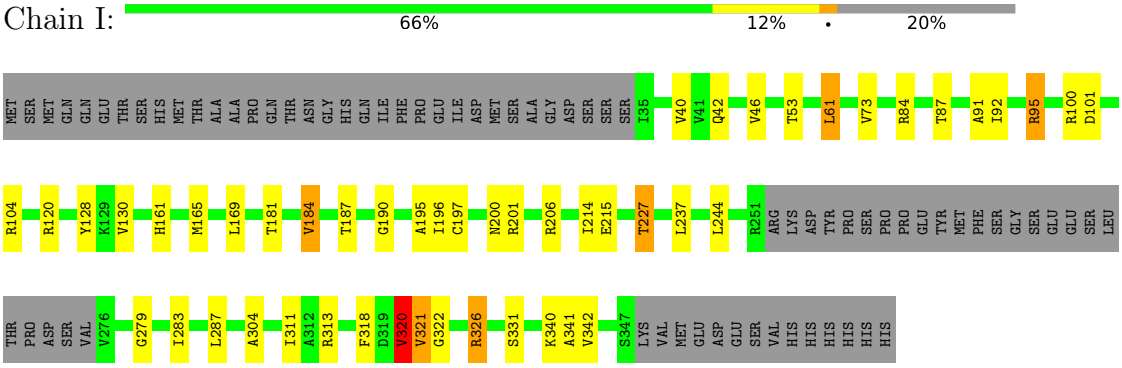
- Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

Chain J:



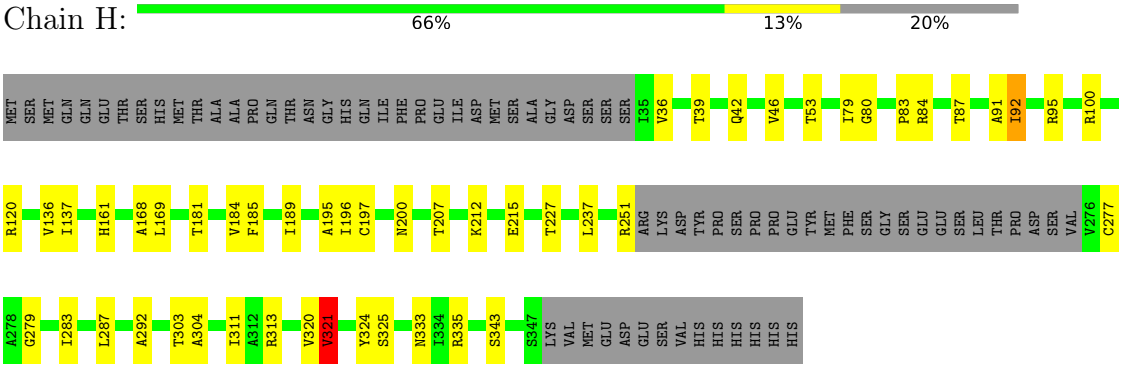
- Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

Chain I:



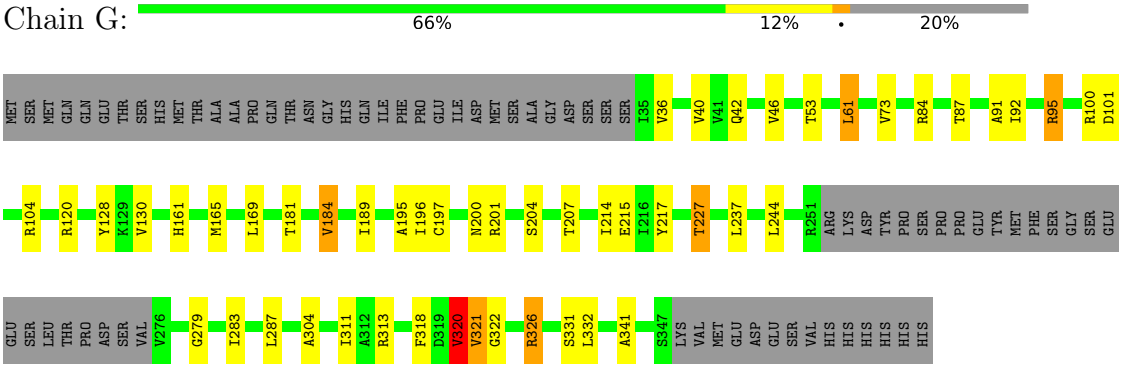
- Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

Chain H:



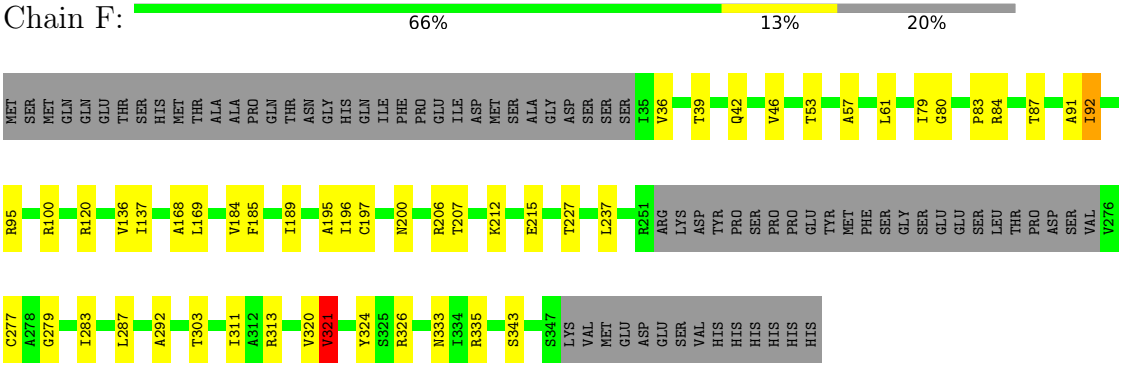
• Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

Chain G:



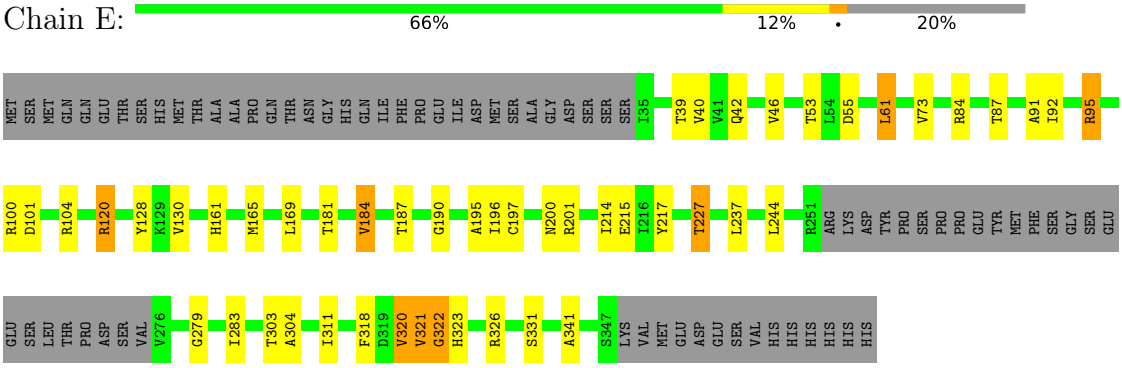
• Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

Chain F:



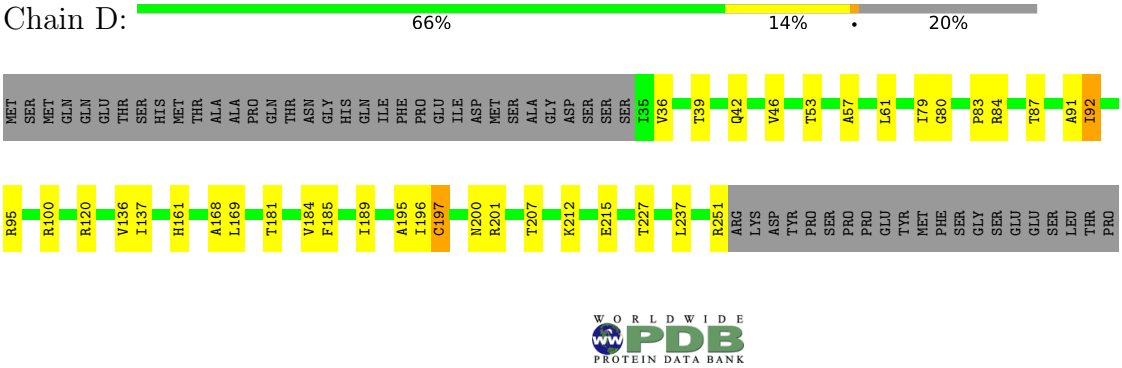
• Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

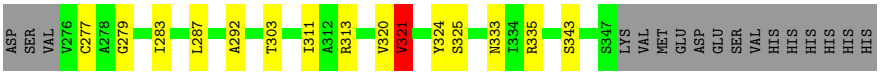
Chain E:



• Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

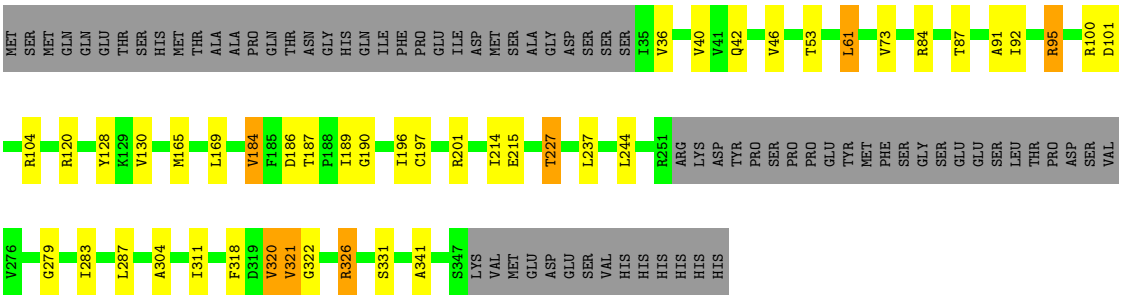
Chain D:





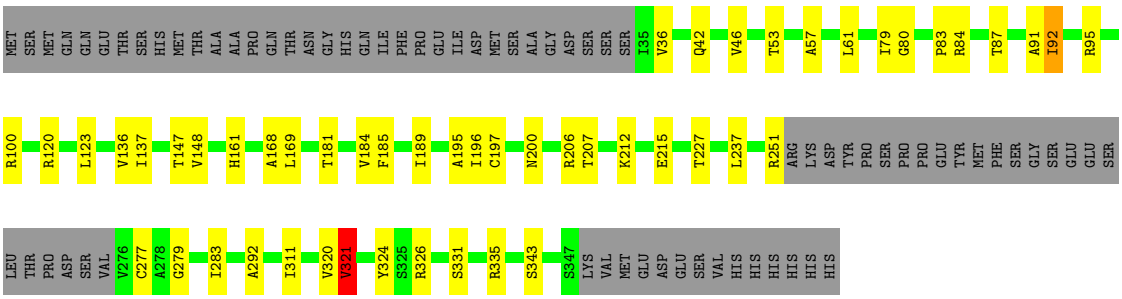
• Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

Chain C:



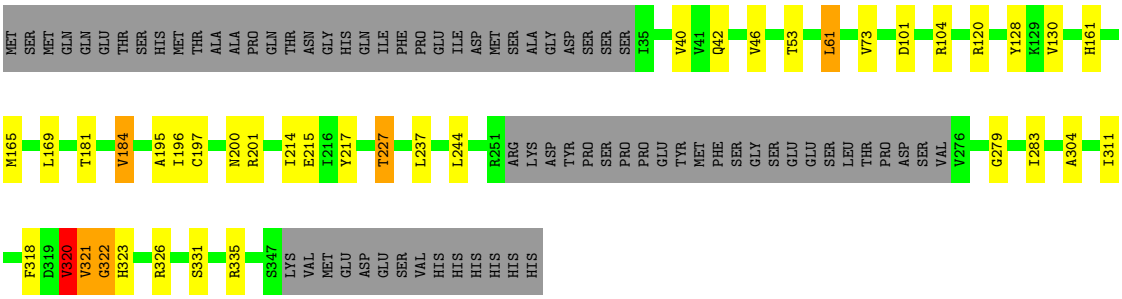
• Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

Chain B:



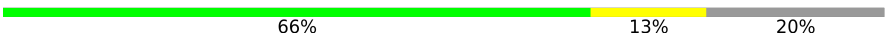
• Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

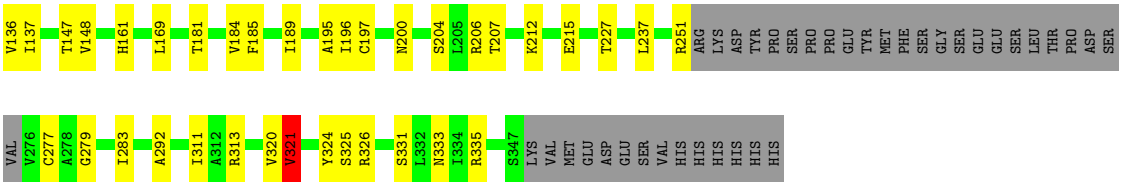
Chain A:



• Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

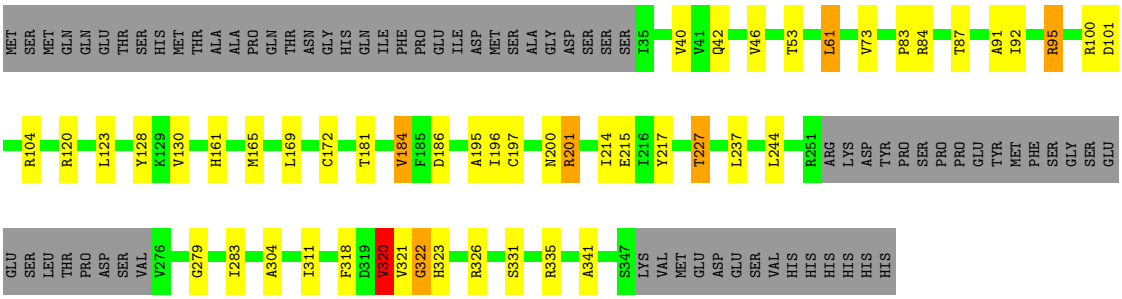
Chain L:





• Molecule 1: Bifunctional nitrilase/nitrile hydratase NIT4

Chain K:



4 Experimental information (i)

Property	Value	Source
EM reconstruction method	HELICAL	Depositor
Imposed symmetry	HELICAL, twist=-72.98°, rise=17.48 Å, axial sym=C1	Depositor
Number of segments used	133106	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	45.5	Depositor
Minimum defocus (nm)	750	Depositor
Maximum defocus (nm)	2000	Depositor
Magnification	Not provided	
Image detector	GATAN K2 SUMMIT (4k x 4k)	Depositor

5 Model quality

5.1 Standard geometry

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).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	#$\|Z\|$ >5	RMSZ	#$\|Z\|$ >5
1	A	0.43	0/2276	0.79	0/3081
1	B	0.54	1/2276 (0.0%)	0.86	4/3081 (0.1%)
1	C	0.44	0/2276	0.79	1/3081 (0.0%)
1	D	0.57	1/2276 (0.0%)	0.84	1/3081 (0.0%)
1	E	0.44	0/2276	0.79	0/3081
1	F	0.46	0/2276	0.85	3/3081 (0.1%)
1	G	0.44	0/2276	0.80	1/3081 (0.0%)
1	H	0.51	1/2276 (0.0%)	0.85	1/3081 (0.0%)
1	I	0.44	0/2276	0.81	1/3081 (0.0%)
1	J	0.46	1/2276 (0.0%)	0.84	4/3081 (0.1%)
1	K	0.44	0/2276	0.80	1/3081 (0.0%)
1	L	0.47	2/2276 (0.1%)	0.84	3/3081 (0.1%)
All	All	0.47	6/27312 (0.0%)	0.82	20/36972 (0.1%)

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 outliers	#Planarity outliers
1	A	0	3
1	C	0	3
1	D	0	1
1	E	0	3
1	G	0	3
1	I	0	3
1	K	0	3
1	L	0	1
All	All	0	20

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

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	D	251	ARG	C-O	16.22	1.54	1.23
1	B	251	ARG	C-O	13.75	1.49	1.23
1	H	251	ARG	C-O	10.87	1.44	1.23
1	L	251	ARG	C-O	6.64	1.35	1.23
1	J	251	ARG	C-O	5.64	1.34	1.23

The worst 5 of 20 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	B	251	ARG	CA-C-O	7.85	136.59	120.10
1	B	206	ARG	NE-CZ-NH2	-6.48	117.06	120.30
1	D	207	THR	OG1-CB-CG2	-6.03	96.14	110.00
1	L	206	ARG	NE-CZ-NH2	-5.98	117.31	120.30
1	F	207	THR	OG1-CB-CG2	-5.95	96.31	110.00

There are no chirality outliers.

5 of 20 planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	G	201	ARG	Sidechain
1	G	320	VAL	Peptide
1	I	201	ARG	Sidechain
1	I	320	VAL	Peptide
1	I	326	ARG	Sidechain

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 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.

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	2226	0	2224	27	0
1	B	2226	0	2224	41	0
1	C	2226	0	2224	35	0
1	D	2226	0	2224	42	0
1	E	2226	0	2224	40	0
1	F	2226	0	2224	40	0
1	G	2226	0	2224	39	0
1	H	2226	0	2224	43	0
1	I	2226	0	2224	42	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	J	2226	0	2224	40	0
1	K	2226	0	2224	40	0
1	L	2226	0	2224	34	0
All	All	26712	0	26688	362	0

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 7.

The worst 5 of 362 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)
1:J:168:ALA:HB2	1:K:95:ARG:HH21	1.29	0.97
1:J:168:ALA:HB2	1:K:95:ARG:NH2	1.91	0.84
1:D:196:ILE:HG22	1:D:197:CYS:H	1.42	0.84
1:H:196:ILE:HG22	1:H:197:CYS:H	1.43	0.84
1:J:92:ILE:HG21	1:K:84:ARG:HA	1.60	0.84

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	Percentiles	
1	A	285/361 (79%)	236 (83%)	47 (16%)	2 (1%)	22	55
1	B	285/361 (79%)	238 (84%)	45 (16%)	2 (1%)	22	55
1	C	285/361 (79%)	238 (84%)	45 (16%)	2 (1%)	22	55
1	D	285/361 (79%)	236 (83%)	46 (16%)	3 (1%)	14	44
1	E	285/361 (79%)	235 (82%)	48 (17%)	2 (1%)	22	55
1	F	285/361 (79%)	240 (84%)	43 (15%)	2 (1%)	22	55
1	G	285/361 (79%)	238 (84%)	45 (16%)	2 (1%)	22	55

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
1	H	285/361 (79%)	236 (83%)	47 (16%)	2 (1%)	22	55
1	I	285/361 (79%)	233 (82%)	50 (18%)	2 (1%)	22	55
1	J	285/361 (79%)	236 (83%)	47 (16%)	2 (1%)	22	55
1	K	285/361 (79%)	236 (83%)	47 (16%)	2 (1%)	22	55
1	L	285/361 (79%)	238 (84%)	45 (16%)	2 (1%)	22	55
All	All	3420/4332 (79%)	2840 (83%)	555 (16%)	25 (1%)	26	55

5 of 25 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	J	321	VAL
1	I	321	VAL
1	H	321	VAL
1	G	321	VAL
1	F	321	VAL

5.3.2 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 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	Percentiles	
1	A	233/299 (78%)	226 (97%)	7 (3%)	41	68
1	B	233/299 (78%)	229 (98%)	4 (2%)	60	80
1	C	233/299 (78%)	226 (97%)	7 (3%)	41	68
1	D	233/299 (78%)	229 (98%)	4 (2%)	60	80
1	E	233/299 (78%)	226 (97%)	7 (3%)	41	68
1	F	233/299 (78%)	229 (98%)	4 (2%)	60	80
1	G	233/299 (78%)	225 (97%)	8 (3%)	37	65
1	H	233/299 (78%)	229 (98%)	4 (2%)	60	80
1	I	233/299 (78%)	224 (96%)	9 (4%)	32	61
1	J	233/299 (78%)	229 (98%)	4 (2%)	60	80
1	K	233/299 (78%)	225 (97%)	8 (3%)	37	65

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	L	233/299 (78%)	229 (98%)	4 (2%)	60 80
All	All	2796/3588 (78%)	2726 (98%)	70 (2%)	50 72

5 of 70 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	320	VAL
1	L	120	ARG
1	K	130	VAL
1	G	320	VAL
1	G	227	THR

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 16 such sidechains are listed below:

Mol	Chain	Res	Type
1	L	200	ASN
1	A	200	ASN
1	E	200	ASN
1	B	200	ASN
1	F	234	HIS

5.3.3 RNA (i)

There are no RNA molecules in this entry.

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)

There are no ligands in this entry.

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

This section contains visualisations of the EMDB entry EMD-0320. 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

This section was not generated.

6.2 Central slices

This section was not generated.

6.3 Largest variance slices

This section was not generated.

6.4 Orthogonal standard-deviation projections (False-color)

This section was not generated.

6.5 Orthogonal surface views

This section was not generated.

6.6 Mask visualisation

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

7 Map analysis

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

7.1 Map-value distribution

This section was not generated.

7.2 Volume estimate versus contour level

This section was not generated.

7.3 Rotationally averaged power spectrum

This section was not generated. The rotationally averaged power spectrum had issues being displayed.

8 Fourier-Shell correlation (i)

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

9 Map-model fit

This section was not generated.