W O R L D W I D E
wwPDB
P R O T E I N   D A T A   B A N K

wwPDB X-ray Structure Validation Summary Report (i)

Jan 15, 2024 – 02:32 pm GMT

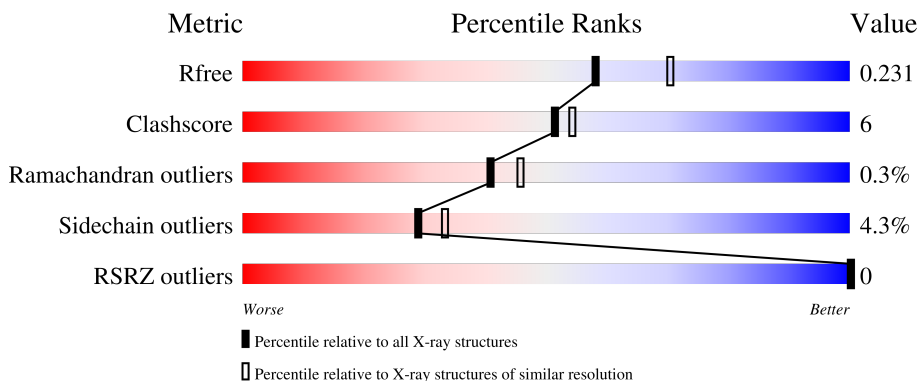|		
PDB ID	:	6TFD
Title	:	Crystal structure of nitrite and NO bound three-domain copper-containing nitrite reductase from Hyphomicrobium denitrificans strain 1NES1
Authors	:	Sasaki, D.; Watanabe, T.F.; Eady, R.R.; Garratt, R.C.; Antonyuk, S.V.; Hasnain, S.S.
Deposited on	:	2019-11-13
Resolution	:	2.25 Å(reported)

1 Overall quality at a glance ⓘ

The following experimental techniques were used to determine the structure:
X-RAY DIFFRACTION

The reported resolution of this entry is 2.25 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	Percentile Ranks	Value
Rfree		0.231
Clashscore		6
Ramachandran outliers		0.3%
Sidechain outliers		4.3%
RSRZ outliers		0

Worse   *Better*

▮ Percentile relative to all X-ray structures
▯ Percentile relative to X-ray structures of similar resolution

Metric	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
R_{free}	130704	1377 (2.26-2.26)
Clashscore	141614	1487 (2.26-2.26)
Ramachandran outliers	138981	1449 (2.26-2.26)
Sidechain outliers	138945	1450 (2.26-2.26)
RSRZ outliers	127900	1356 (2.26-2.26)

The table below summarises the geometric issues observed across the polymeric chains and their fit to the electron density. The red, orange, yellow and green segments of the lower 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% The upper red bar (where present) indicates the fraction of residues that have poor fit to the electron density. The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	456	80% 11% • 7%
1	B	456	80% 12% • 7%
1	C	456	83% 10% 7%

2 Entry composition ⓘ

There are 5 unique types of molecules in this entry. The entry contains 10281 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, 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 Copper-containing nitrite reductase.

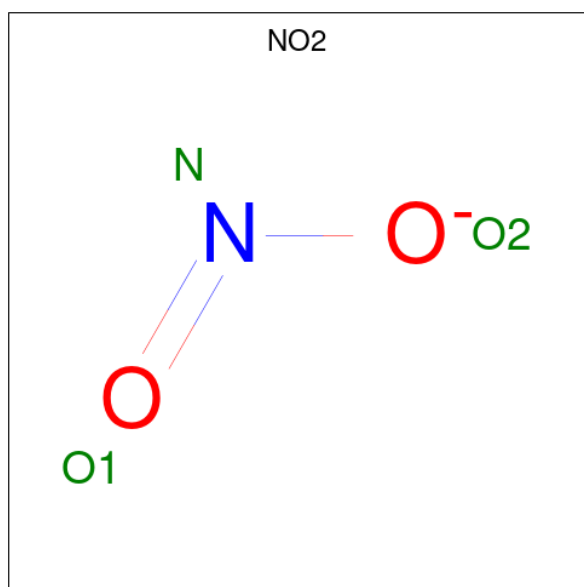| Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	423	Total 3218	C 2045	N 553	O 610	S 10	0	1	0
1	B	423	Total 3195	C 2032	N 546	O 607	S 10	0	0	0
1	C	423	Total 3212	C 2042	N 550	O 610	S 10	0	1	0

There are 21 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1	MET	-	initiating methionine	UNP N0B9M5
A	451	GLU	-	expression tag	UNP N0B9M5
A	452	ASN	-	expression tag	UNP N0B9M5
A	453	LEU	-	expression tag	UNP N0B9M5
A	454	TYR	-	expression tag	UNP N0B9M5
A	455	PHE	-	expression tag	UNP N0B9M5
A	456	GLN	-	expression tag	UNP N0B9M5
B	1	MET	-	initiating methionine	UNP N0B9M5
B	451	GLU	-	expression tag	UNP N0B9M5
B	452	ASN	-	expression tag	UNP N0B9M5
B	453	LEU	-	expression tag	UNP N0B9M5
B	454	TYR	-	expression tag	UNP N0B9M5
B	455	PHE	-	expression tag	UNP N0B9M5
B	456	GLN	-	expression tag	UNP N0B9M5
C	1	MET	-	initiating methionine	UNP N0B9M5
C	451	GLU	-	expression tag	UNP N0B9M5
C	452	ASN	-	expression tag	UNP N0B9M5
C	453	LEU	-	expression tag	UNP N0B9M5
C	454	TYR	-	expression tag	UNP N0B9M5
C	455	PHE	-	expression tag	UNP N0B9M5
C	456	GLN	-	expression tag	UNP N0B9M5

- Molecule 2 is COPPER (II) ION (three-letter code: CU) (formula: Cu).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
2	A	3	Total Cu 3 3		0	0
2	B	3	Total Cu 3 3		0	0
2	C	3	Total Cu 3 3		0	0

- Molecule 3 is NITRITE ION (three-letter code: NO2) (formula: NO_2) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
3	A	1	Total N O 3 1 2			0	0
3	A	1	Total N O 3 1 2			0	0

- Molecule 4 is NITRIC OXIDE (three-letter code: NO) (formula: NO) (labeled as "Ligand of Interest" by depositor).

NO
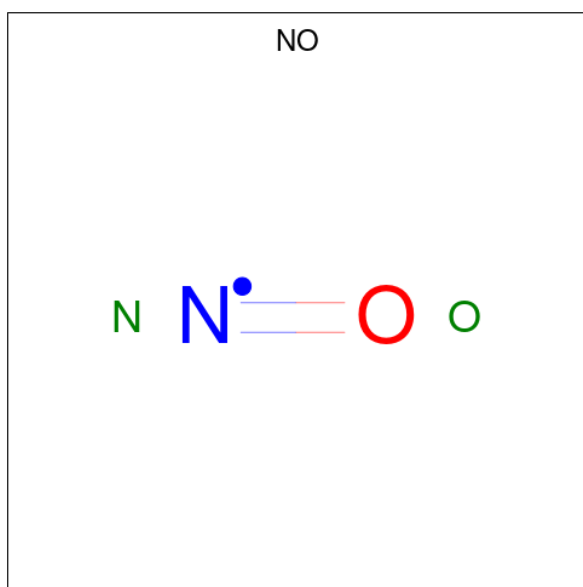
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
4	B	1	Total 2	N 1	O 1	0	0

- Molecule 5 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
5	A	240	Total 240	O 240	0	0
5	B	211	Total 211	O 211	0	0
5	C	188	Total 188	O 188	0	0

3 Residue-property plots ⓘ

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 electron 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 dot above a residue indicates a poor fit to the electron density (RSRZ > 2). 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.
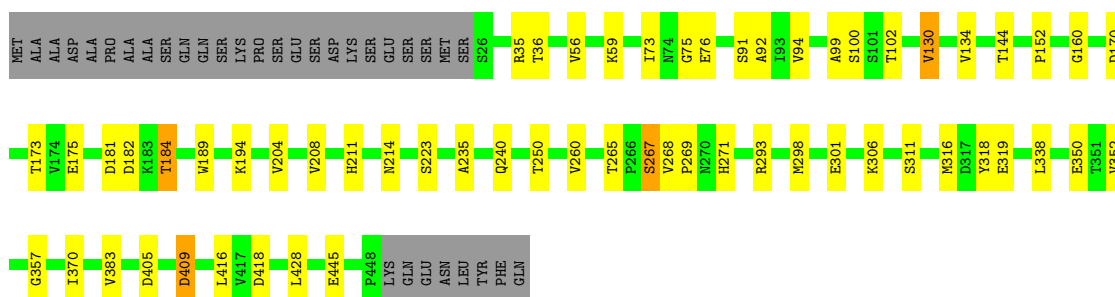
- Molecule 1: Copper-containing nitrite reductase
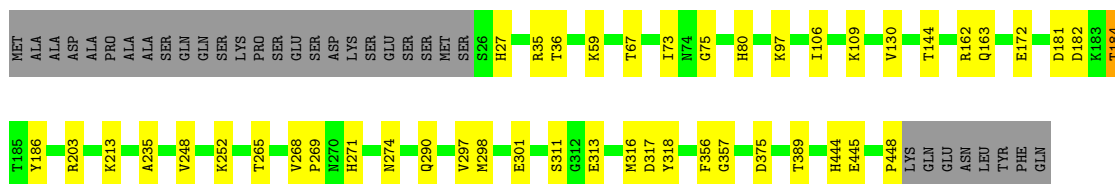
Chain A:
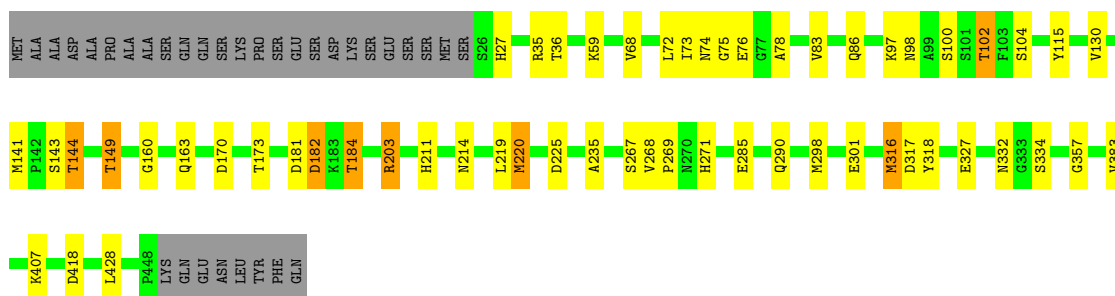
- Molecule 1: Copper-containing nitrite reductase

Chain B:

- Molecule 1: Copper-containing nitrite reductase

Chain C:

4 Data and refinement statistics ⓘ

Property	Value	Source
Space group	P 65 2 2	Depositor
Cell constants a, b, c, α, β, γ	77.72Å 77.72Å 758.20Å 90.00° 90.00° 120.00°	Depositor
Resolution (Å)	126.37 – 2.25 126.37 – 2.25	Depositor EDS
% Data completeness (in resolution range)	100.0 (126.37-2.25) 100.0 (126.37-2.25)	Depositor EDS
R_{merge}	0.22	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	1.80 (at 2.25Å)	Xtriage
Refinement program	REFMAC 5.8.0238	Depositor
R, R_{free}	0.172 , 0.227 0.181 , 0.231	Depositor DCC
R_{free} test set	3336 reflections (4.98%)	wwPDB-VP
Wilson B-factor (Å^2)	48.1	Xtriage
Anisotropy	0.043	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.32 , 37.7	EDS
L-test for twinning[2]	$< \|L\| > = 0.48, < L^2 > = 0.31$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.97	EDS
Total number of atoms	10281	wwPDB-VP
Average B, all atoms (Å^2)	54.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.45% of the height of the origin peak. No significant pseudotranslation is detected.*

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[1]Intensities estimated from amplitudes.
[2]Theoretical values of $< \|L\| >$, $< L^2 >$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

5 Model quality ⓘ

5.1 Standard geometry ⓘ

Bond lengths and bond angles in the following residue types are not validated in this section: NO2, CU, NO

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	0.72	0/3298	0.88	0/4491
1	B	0.72	0/3275	0.87	0/4463
1	C	0.74	0/3292	0.87	1/4484 (0.0%)
All	All	0.73	0/9865	0.88	1/13438 (0.0%)

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	1
1	B	0	1
All	All	0	2

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	C	448	PRO	CA-C-O	5.10	132.44	120.20

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	160	GLY	Peptide
1	B	160	GLY	Peptide

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	3218	0	3149	43	0
1	B	3195	0	3114	35	0
1	C	3212	0	3138	32	0
2	A	3	0	0	0	0
2	B	3	0	0	0	0
2	C	3	0	0	0	0
3	A	6	0	0	1	0
4	B	2	0	0	1	0
5	A	240	0	0	3	0
5	B	211	0	0	3	0
5	C	188	0	0	8	0
All	All	10281	0	9401	107	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 6.

The worst 5 of 107 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:C:181:ASP:HB3	1:C:184:THR:HG23	1.48	0.95
1:A:327:GLU:OE1	5:A:601:HOH:O	1.85	0.93
1:A:316:MET:HE1	1:A:318:TYR:HA	1.57	0.86
1:A:181:ASP:HB3	1:A:184:THR:HG23	1.61	0.83
1:A:144:THR:HB	5:A:671:HOH:O	1.88	0.73

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 X-ray entries followed by that with respect to entries of similar resolution.

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	422/456 (92%)	410 (97%)	11 (3%)	1 (0%)	47	55
1	B	421/456 (92%)	410 (97%)	10 (2%)	1 (0%)	47	55
1	C	422/456 (92%)	410 (97%)	10 (2%)	2 (0%)	29	29
All	All	1265/1368 (92%)	1230 (97%)	31 (2%)	4 (0%)	41	46

All (4) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	235	ALA
1	C	235	ALA
1	B	235	ALA
1	C	162	ARG

5.3.2 Protein sidechains ⓘ

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all X-ray entries followed by that with respect to entries of similar resolution.

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	344/371 (93%)	327 (95%)	17 (5%)	25	27
1	B	340/371 (92%)	323 (95%)	17 (5%)	24	26
1	C	343/371 (92%)	332 (97%)	11 (3%)	39	47
All	All	1027/1113 (92%)	982 (96%)	45 (4%)	29	32

5 of 45 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	267	SER
1	C	109	LYS
1	B	301	GLU
1	B	383	VAL
1	C	163	GLN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 10

such sidechains are listed below:

Mol	Chain	Res	Type
1	C	240	GLN
1	C	274	ASN
1	C	444	HIS
1	A	444	HIS
1	B	274	ASN

5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

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

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

5.5 Carbohydrates ⓘ

There are no monosaccharides in this entry.

5.6 Ligand geometry ⓘ

Of 12 ligands modelled in this entry, 9 are monoatomic - leaving 3 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).

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	#$|Z| > 2$	Counts	RMSZ	#$|Z| > 2$
4	NO	B	504	2	0,1,1	-	-	-		
3	NO2	A	504	2	1,2,2	0.87	0	0,1,1	-	-
3	NO2	A	505	2	1,2,2	0.46	0	0,1,1	-	-

There are no bond length outliers.

There are no bond angle outliers.
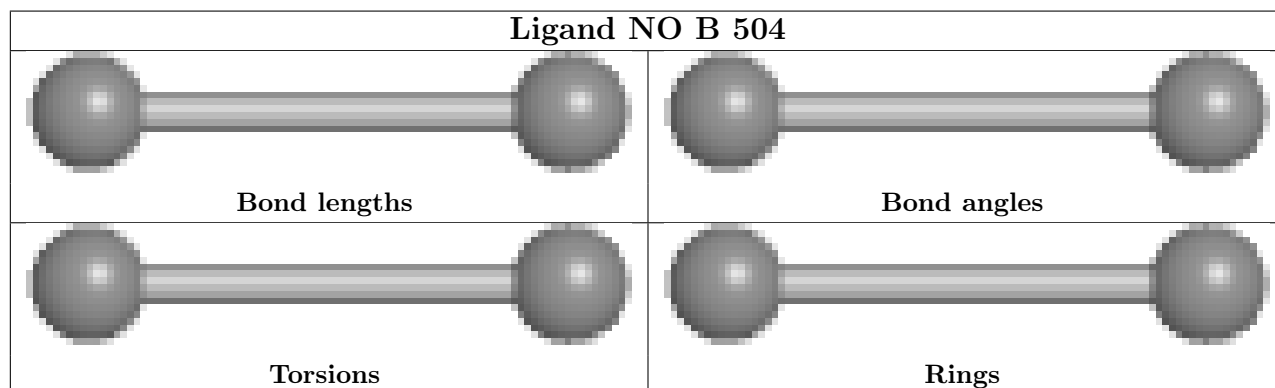
There are no chirality outliers.
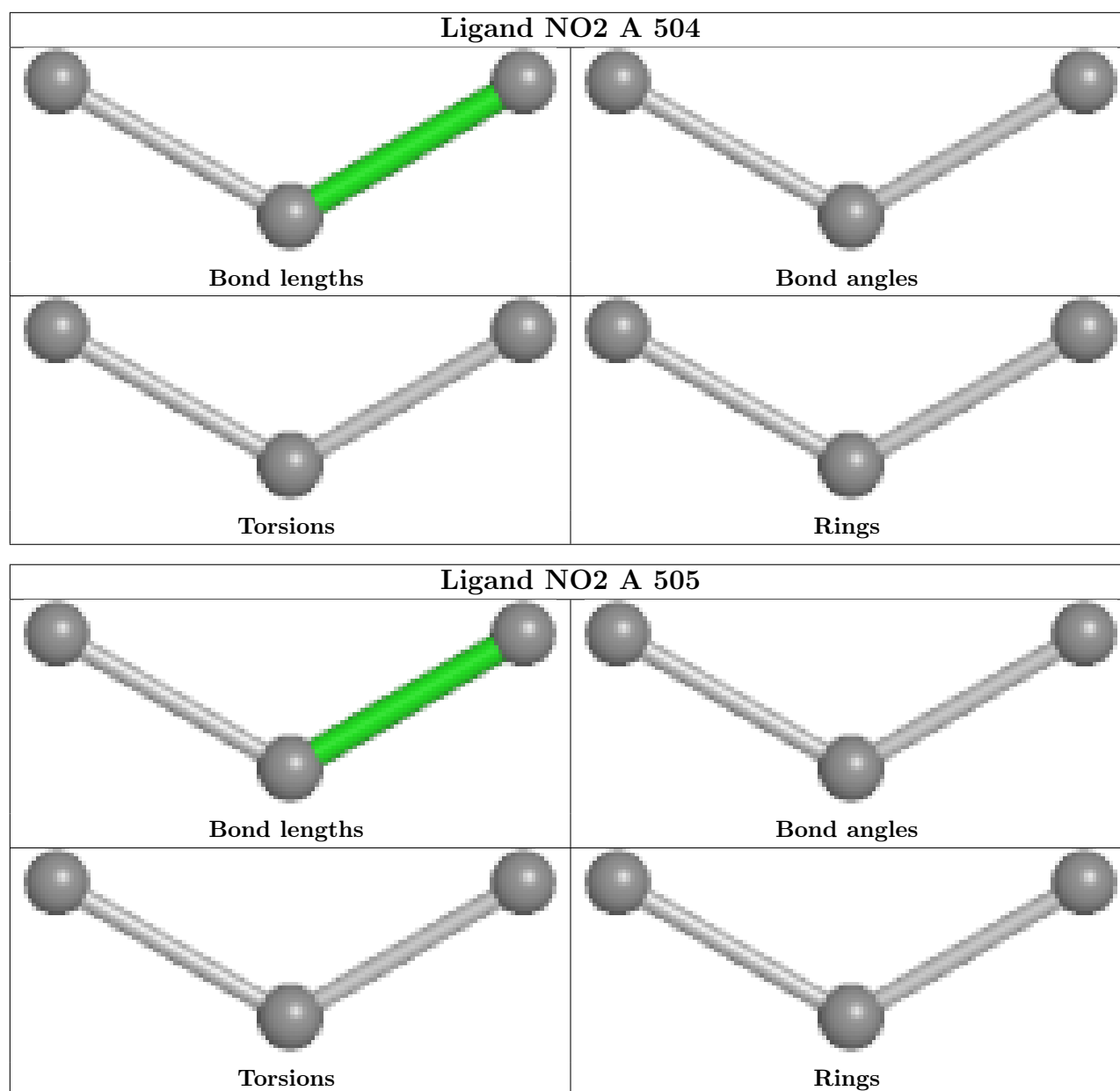
There are no torsion outliers.

There are no ring outliers.

2 monomers are involved in 2 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	B	504	NO	1	0
3	A	504	NO2	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.

Ligand NO B 504	
Bond lengths	Bond angles
Torsions	Rings

Ligand NO2 A 504

Bond lengths

Bond angles

Torsions

Rings

Ligand NO2 A 505

Bond lengths

Bond angles

Torsions

Rings

5.7 Other polymers ⓘ

There are no such residues in this entry.

5.8 Polymer linkage issues ⓘ

There are no chain breaks in this entry.

6 Fit of model and data ⓘ

6.1 Protein, DNA and RNA chains ⓘ

In the following table, the column labelled '#RSRZ> 2' contains the number (and percentage) of RSRZ outliers, followed by percent RSRZ outliers for the chain as percentile scores relative to all X-ray entries and entries of similar resolution. The OWAB column contains the minimum, median, 95th percentile and maximum values of the occupancy-weighted average B-factor per residue. The column labelled 'Q< 0.9' lists the number of (and percentage) of residues with an average occupancy less than 0.9.

Mol	Chain	Analysed	<RSRZ>	#RSRZ>2			OWAB(Å^2)	Q<0.9
1	A	423/456 (92%)	-0.50	0	100	100	36, 49, 76, 100	0
1	B	423/456 (92%)	-0.47	0	100	100	40, 53, 79, 112	0
1	C	423/456 (92%)	-0.48	0	100	100	38, 53, 76, 104	0
All	All	1269/1368 (92%)	-0.48	0	100	100	36, 52, 78, 112	0

There are no RSRZ outliers to report.

6.2 Non-standard residues in protein, DNA, RNA chains ⓘ

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

6.3 Carbohydrates ⓘ

There are no monosaccharides in this entry.

6.4 Ligands ⓘ

In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95th percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists the number of atoms with occupancy less than 0.9.
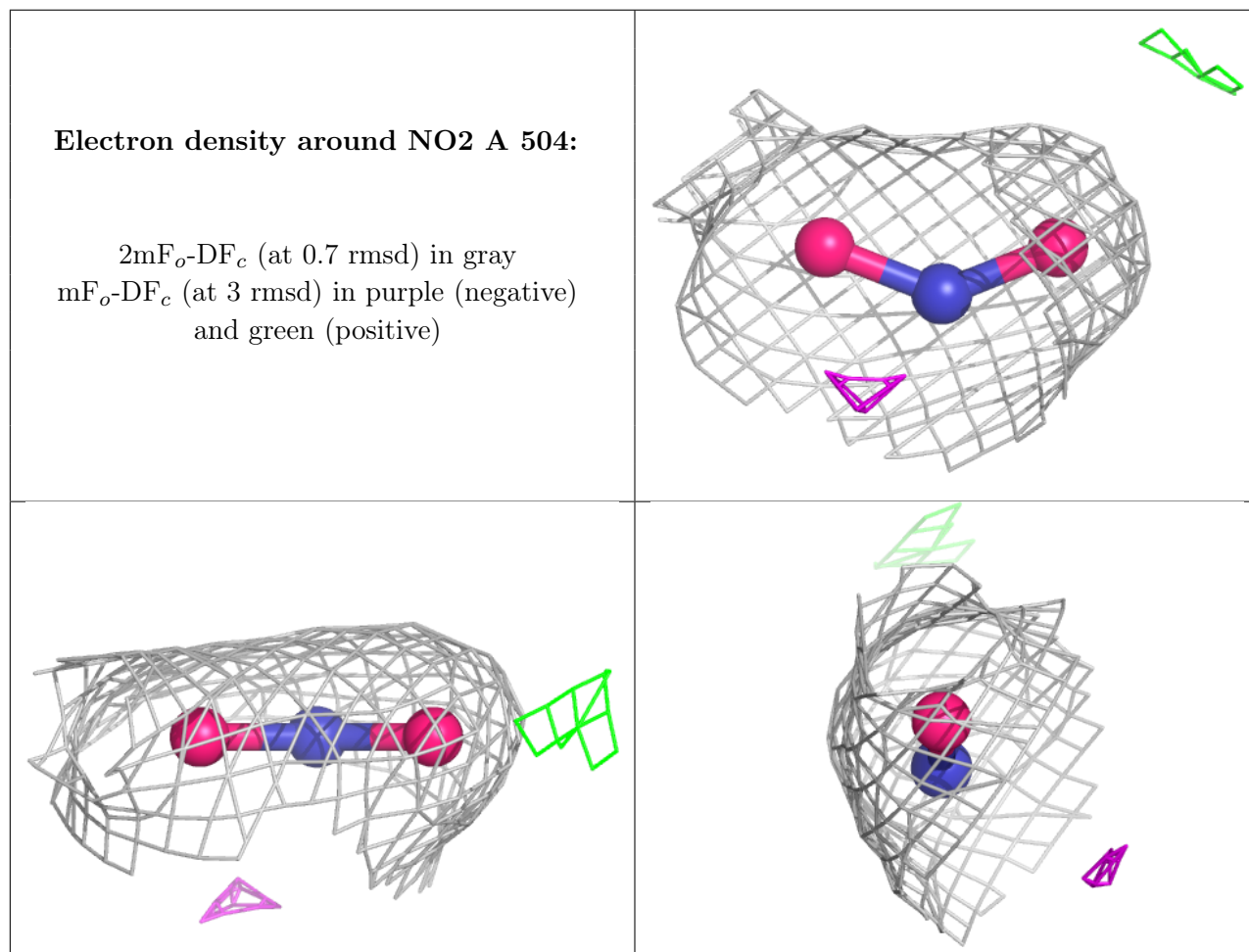
Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å^2)	Q<0.9
3	NO2	A	504	3/3	0.99	0.11	52,52,66,68	0
2	CU	A	502	1/1	1.00	0.09	50,50,50,50	0
2	CU	A	503	1/1	1.00	0.10	48,48,48,48	0
2	CU	B	501	1/1	1.00	0.09	49,49,49,49	0
2	CU	B	502	1/1	1.00	0.10	49,49,49,49	0

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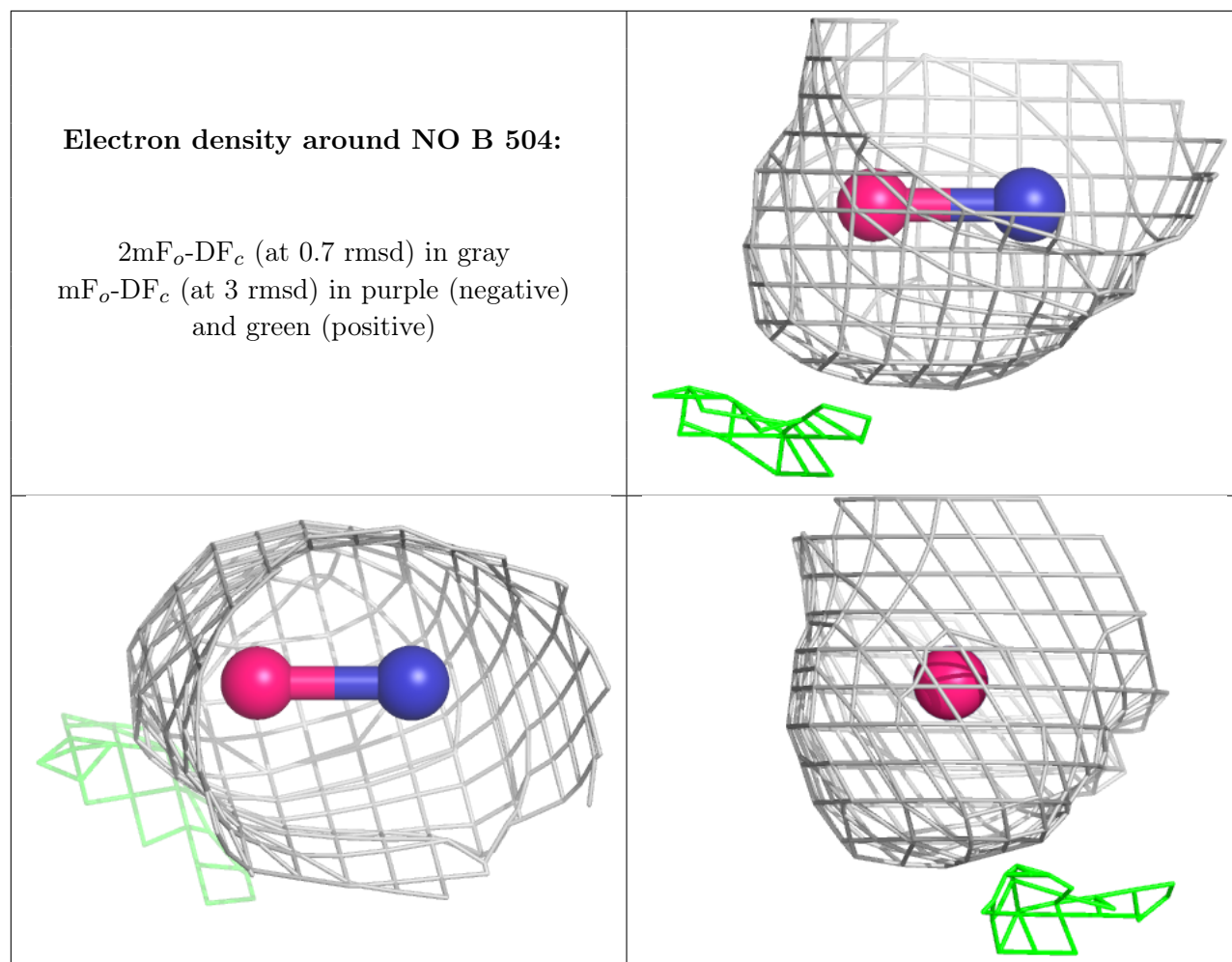| Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å^2)	Q<0.9
2	CU	B	503	1/1	1.00	0.10	48,48,48,48	0
2	CU	C	501	1/1	1.00	0.10	48,48,48,48	0
2	CU	C	502	1/1	1.00	0.13	49,49,49,49	0
2	CU	C	503	1/1	1.00	0.10	49,49,49,49	0
2	CU	A	501	1/1	1.00	0.12	45,45,45,45	0
3	NO2	A	505	3/3	1.00	0.10	47,47,48,55	0
4	NO	B	504	2/2	1.00	0.11	52,52,52,57	0

The following is a graphical depiction of the model fit to experimental electron density of all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the geometry validation Tables will also be included. Each fit is shown from different orientation to approximate a three-dimensional view.

Electron density around NO2 A 504:

2mF$_o$-DF$_c$ (at 0.7 rmsd) in gray
mF$_o$-DF$_c$ (at 3 rmsd) in purple (negative)
and green (positive)

Electron density around NO2 A 505:

2mF$_o$-DF$_c$ (at 0.7 rmsd) in gray
mF$_o$-DF$_c$ (at 3 rmsd) in purple (negative)
and green (positive)

Electron density around NO B 504:

$2mF_o$-DF_c (at 0.7 rmsd) in gray
mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)

6.5 Other polymers ⓘ

There are no such residues in this entry.