SUPPLEMENTARY INFORMATION

Structure of the membrane-intrinsic nitric oxide reductase from *Roseobacter denitrificans*

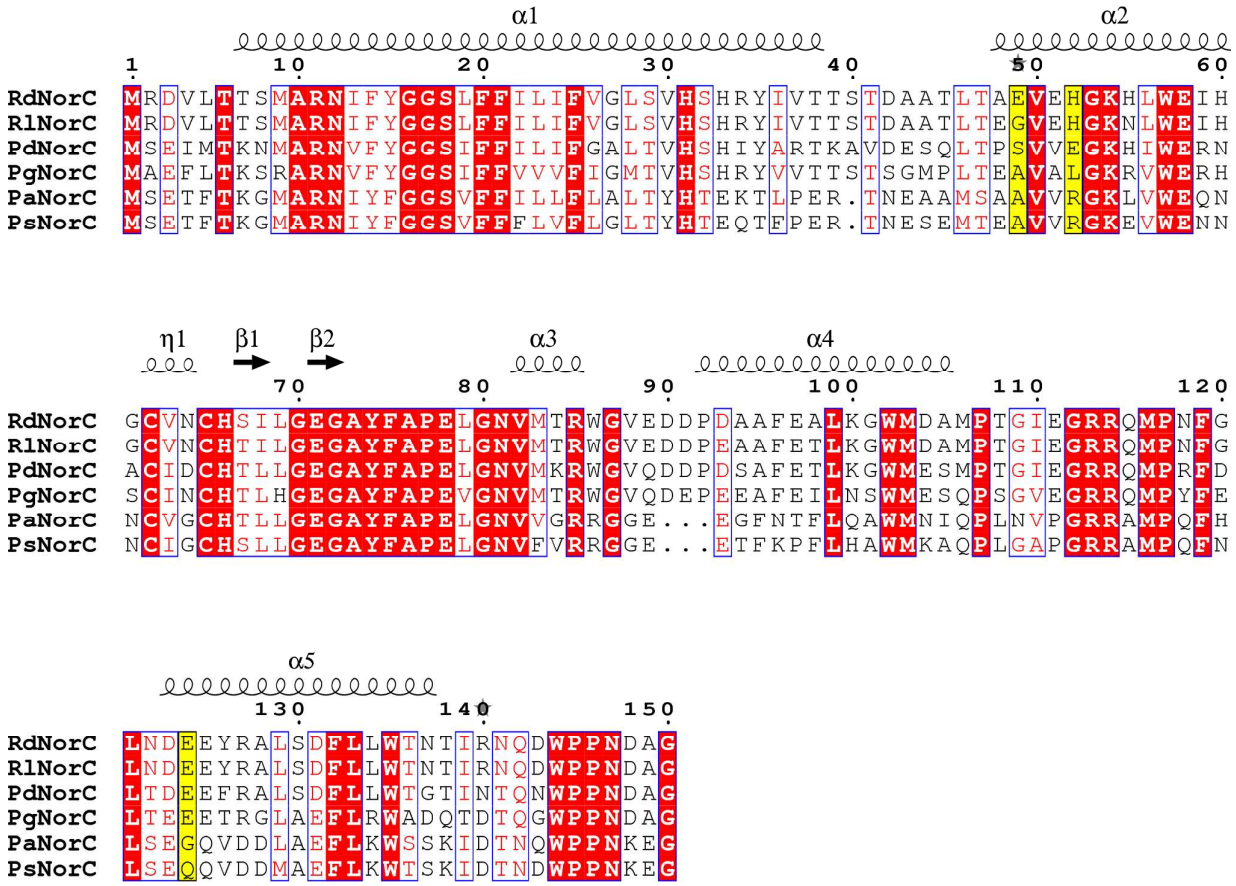
Allister Crow, Yuji Matsuda, Hiroyuki Arata, Arthur Oubrie

Supplemental Table 1: Crystallographic data and refinement statistics

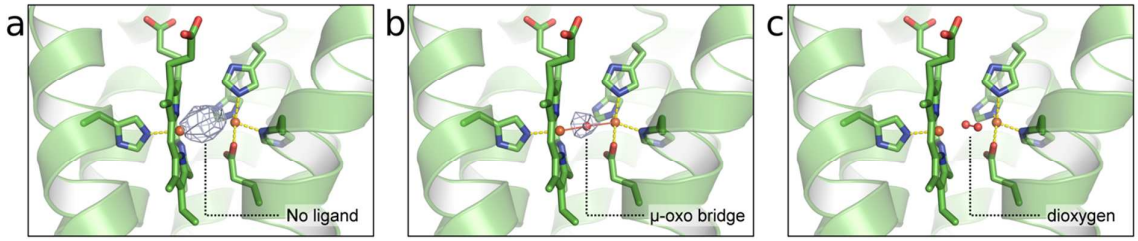
Data Collection		
Beam line	ESRF ID23-1	ESRF ID23-1
Wavelength (Å)	0.9762	1.7385
Crystal Parameters		
Space group	P 2 2_1 2_1	P 2 2_1 2_1
Unit cell dimensions (Å)	*a* = 86.2, *b* = 98.8, c = 126.8	*a* = 85.3, *b* = 98.7, *c* = 125.6
Unit cell angles (°)	90, 90, 90	90, 90, 90
Dataset Statistics		
Resolution range (Å)	53.36 – 2.85 (3.00 – 2.85)	51.30-3.60 (3.79-3.60)
Unique reflections	25,236 (3,124)	12,977 (1,860)
R_{sym}	0.102 (0.362)	0.112 (0.373)
$I/\sigma(I)$	13.3 (3.4)	7.9 (2.9)
Completeness (%)	97.2 (85.7)	98.8 (99.5)
Multiplicity	6.7 (4.7)	3.4 (3.4)
Refinement		
Number of reflections	23,917	-
R_{work}	0.1885	-
R_{free}	0.2435	-
Rms bond lengths (Å)	0.0091	-
Rms bond angles (°)	1.5883	-
Model statistics		
Ramachandran outliers	1 (0.2%)	-
Number of Atoms (Protein)	4,726	-
Number of Atoms (Ligands/ions)	134	-
Number of Atoms (Waters)	15	-
B-factors (Protein) (Å^2)	45	-
B-factors (Ligands) (Å^2)	36	-
B-factors (Waters) (Å^2)	40	-

	λ = 1.7385 Å Dataset		λ = 0.9762 Å Dataset		Ratio		Metal
	Observed	*Theoretical*	*Observed*	*Theoretical*	*Observed*	*Theoretical*	
Heme *c*	4.1	3.9	1.5	1.5	0.4	0.4	Fe
Heme *b*	4.2	3.9	1.8	1.5	0.4	0.4	Fe
Heme b_3	3.4	3.9	1.2	1.5	0.3	0.4	Fe
Active site metal	4.0	3.9	1.5	1.5	0.4	0.4	Fe
NorC metal	0.5	0.7	1.1	2.2	2.6	3.0	Cu
Bridging metal	1.3	1.6	0.4	0.6	0.3	0.4	Ca

Supplementary Table 2. Integrated anomalous scattering map values for each metal site in RdNOR. All values in electrons. Observed values correspond to the integrated peaks from the anomalous scattering maps. Theoretical *f"* values correspond to the assigned metal given in the rightmost column.



Supplemental Figure 1. Multiple sequence alignment of the RdNOR small subunit and five key homologues in the region of the small subunit metal site. RdNorC – *Roseobacter denitrificans*; PaNorC – *Pseudomonas aeruginosa*; PdNorC-*Paracoccus denitrificans*; PsNorC-*Pseudomonas stutzeri*; RlNorC-*Roseobacter litoralis*; PgNorC-*Phaeobacter gallaeciensis.* Secondary structure elements from the structure of RdNOR are shown immediately above the alignment. Locations of residues aligning with those coordinating the small subunit metal site in RdNOR are highlighted *gold*.



Supplemental Figure 2. Alternative models for the ligand bridging the active site site di-iron centre. The residual electron density for each model is shown as a blue mesh contoured at 5 σ. (**a**) Active site without a bridging ligand. (**b**) A μ-oxo bridge. (**c**) A bridging di-oxygen molecule. Iron atoms are shown as *orange* spheres. Only models with a diatomic ligand properly account for the observed electron density. While dioxygen is shown modelled in c, similar results were obtained for dinitrogen, carbon monoxide, nitric oxide, and a peroxo-bridge.