

Crystal structure of NirF: insights into its role in heme d_1 biosynthesis

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Supplementary Table 1: Plasmids used in this work

Plasmid name	Description	Reference
pUCP20T	<i>Escherichia-Pseudomonas</i> shuttle vector	West <i>et al.</i> [1]
pUCP20T- <i>nirF</i>	<i>nirF</i> gene cloned pUCP20T	Nicke <i>et al.</i> [2]
pET-SUMO	pET19m (modified pET19 (Invitrogen)) with SUMO tag cloned in Nde1 restriction site in frame with 6xHis and TEV site	Lab Collection
pET-SUMO- <i>nirF</i>	<i>nirF</i> without export signal from pUCP20T- <i>nirF</i> cloned in between Nde1 and BamHI restriction site in frame with SUMO tag	This study
pMALX-E	Modified pMAL-c2x (New England Biolabs) containing for crystallization enhanced maltose binding protein	Moon <i>et al.</i> [3]
pMALX-E- <i>nirF</i>	<i>nirF</i> without export signal from pUCP20T- <i>nirF</i> cloned in between Nhe1 and HindIII restriction site in frame with MBP	This study
pUCP20T- <i>nirF</i> ^{R21A}	pUCP20T- <i>nirF</i> with R21 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{H48A}	pUCP20T- <i>nirF</i> with H48 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{S50A}	pUCP20T- <i>nirF</i> with S50 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{R65A}	pUCP20T- <i>nirF</i> with R65 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{D185A}	pUCP20T- <i>nirF</i> with D185 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{Y234A}	pUCP20T- <i>nirF</i> with Y234 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{K235A}	pUCP20T- <i>nirF</i> with K235 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{H238A}	pUCP20T- <i>nirF</i> with H238 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{E240A}	pUCP20T- <i>nirF</i> with E240 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{H325A}	pUCP20T- <i>nirF</i> with H325 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{R340A}	pUCP20T- <i>nirF</i> with R340 mutated to alanine	This study
pUCP20T- <i>nirF</i> ^{R372A}	pUCP20T- <i>nirF</i> with R372 mutated to alanine	This study
pET-SUMO- <i>nirF</i> ^{R21A}	pET-SUMO- <i>nirF</i> with R21 mutated to alanine	This study
pET-SUMO- <i>nirF</i> ^{H48A}	pET-SUMO- <i>nirF</i> with H48 mutated to alanine	This study
pET-SUMO- <i>nirF</i> ^{R65A}	pET-SUMO- <i>nirF</i> with R65 mutated to alanine	This study
pET-SUMO- <i>nirF</i> ^{D185A}	pET-SUMO- <i>nirF</i> with D185 mutated to alanine	This study
pET-SUMO- <i>nirF</i> ^{K235A}	pET-SUMO- <i>nirF</i> with K235 mutated to alanine	This study
pET-SUMO- <i>nirF</i> ^{H238A}	pET-SUMO- <i>nirF</i> with H238 mutated to alanine	This study
pET-SUMO- <i>nirF</i> ^{R372A}	pET-SUMO- <i>nirF</i> with R372 mutated to alanine	This study

Supplementary Table 2: Strains used in this work

Strain name	Description	Reference
E.coli BL21	<i>Escherichia coli</i> host used for recombinant protein expression	
E.coli XL1-blue	<i>Escherichia coli</i> host used for cloning	
PA01	<i>Pseudomonas aeruginosa</i> PA01	Lab Collection
PA01 RM361	<i>Pseudomonas aeruginosa</i> PA01 <i>nirN::tet</i>	Kawasaki <i>et al.</i> [4]
PA01 RM301	<i>Pseudomonas aeruginosa</i> PA01 <i>nirF::tet</i>	Kawasaki <i>et al.</i> [5]
BL21-Sumo- <i>nirF</i>	<i>E.coli</i> BL21 with pET-SUMO- <i>nirF</i>	This study
BL21-Sumo- <i>nirF</i> ^{R21A}	<i>E.coli</i> BL21 with pET-SUMO- <i>nirF</i> ^{R21A}	This study
BL21-Sumo- <i>nirF</i> ^{H48A}	<i>E.coli</i> BL21 with pET-SUMO- <i>nirF</i> ^{H48A}	This study
BL21-Sumo- <i>nirF</i> ^{R65A}	<i>E.coli</i> BL21 with pET-SUMO- <i>nirF</i> ^{R65A}	This study
BL21-Sumo- <i>nirF</i> ^{D185A}	<i>E.coli</i> BL21 with pET-SUMO- <i>nirF</i> ^{D185A}	This study
BL21-Sumo- <i>nirF</i> ^{K235A}	<i>E.coli</i> BL21 with pET-SUMO- <i>nirF</i> ^{K235A}	This study
BL21-Sumo- <i>nirF</i> ^{H238A}	<i>E.coli</i> BL21 with pET-SUMO- <i>nirF</i> ^{H238A}	This study
BL21-Sumo- <i>nirF</i> ^{R372A}	<i>E.coli</i> BL21 with pET-SUMO- <i>nirF</i> ^{R372A}	This study
PA-wt	PA01 with PUCP20T	This study
PA- Δ <i>nirF</i>	PA01 RM301 with pUCP20T	This study
PA- <i>nirF</i>	PA01 RM301 with pUCP20T- <i>nirF</i>	This study
PA-R21A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{R21A}	This study
PA-H48A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{H48A}	This study
PA-S50A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{S50A}	This study
PA-R65A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{R65A}	This study
PA-D185A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{D185A}	This study
PA-Y234A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{Y234A}	This study
PA-K235A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{K235A}	This study
PA-H238A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{H238A}	This study
PA-E240A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{E240A}	This study
PA-H325A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{H325A}	This study
PA-R340A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{R340A}	This study
PA-R372A	PA01 RM301 with pUCP20T- <i>nirF</i> ^{R372A}	This study

Supplementary Table 3: Primers used in this work; the parts of a primer that match the sequence of NirF are in bold letters. Mismatching base pairs of primers used in mutagenesis are highlighted in bold red.

Primer name	Sequence (5' → 3')
nirF_pET-SUMO_fw	CGCGAACAGATCGGTGGTCATATG ATGAGCCAGCAGCCGC
nirF_pET-SUMO_rv	GCTTTGTTAGCAGCCGGATCCTAG AGTCCGATGTGCTGGGCG
nirF_pMALX-E_fw	CAGACTAATGCGGCCGAGCTAGCAT AGCCAGCAGCCGC
nirF_pMALX-E_rv	CGACGGCCAGTGCCAAGCTT AGAGTCCGATGTGCTGGGCG
nirF_R21A_rv	CCGTCGGCG CTTC GATCAGCACGCCGAG
nirF_R21A_fw	TCGGCGTGCTGATCGAA CGCCG ACGGCAG
nirF_H48A_rv	CACCAGGGAGGCG CGG ACAGGTCGCCG
nirF_H48A_fw	CGGCGACCTGTCC CGC CCTCCCTGGTG
nirF_S50A_rv	GAGAACACCAGGG CGG CGTGGGACAGG
nirF_S50A_fw	CCTGTCCACGCC CGC CTGGTGTTCTC
nirF_R65A_rv	GCCGCCGTCG CGAC CGAATACGTAGGCGTAG
nirF_R65A_fw	TACGCCTACGTATTCGGT CGC GACGGCGGC
nirF_D185A_rv	GGCTGATGAGGGCG CGT AGGGTTGCTTG
nirF_D185A_fw	CAAGCAACCCTACG CGC CCCTCATCAGCC
nirF_Y234A_rv	GGTGCGGCATCTTG CGC ACCGGCAGCTTGC
nirF_Y234A_fw	GCAAGCTGCCGGTG CGC AAGATGCCGCACC
nirF_K235A_rv	CCAGGTGCGGCATC CGT ACACCGGCAGCTTG
nirF_K235A_fw	AGCTGCCGGTGTAC CGC GATGCCGCACCTGG
nirF_H238A_rv	GTCCAGCCCTCCAGG CGC GGCATCTTGTACAC
nirF_H238A_fw	GTGTACAAGATGCCG CGC CTGGAGGGCTGGAC
nirF_E240A_rv	TGGTCCAGCCC CGC AGGTGCGGC
nirF_E240A_fw	GCCGCACCTGG CGG GCTGGACCATC
nirF_H325A_rv	CGCTGAATTCCATG CGC AGTACGCCGGGGC
nirF_H325A_fw	GCCCCGGCGTACTG CGC CATGGAATTCAGCG
nirF_R340A_rv	CTGGTCGGCGTCG CGC ACCGAGATCCAG
nirF_R340A_fw	CTGGATCTCGGTG CGC GACGCCGACCAG
nirF_R372A_rv	CGATGTGCTGGGCG CGT GGCTGAAGAAGATG
nirF_R372A_fw	TCTTCTTCAGCCAC CGC CGCCAGCACATCG

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