

Supplementary Materials

Purification, Characterization, and Ferrous Oxidation Kinetics of Iron Oxidase from *Acidithiobacillus ferridurans*

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Supplementary Text S1

Text S1. The regenerated and synthesized nucleotide gene sequence of the recombinant iron oxidase from *A. ferriidurans* Riv11. The coding sequence of proteins is black and regenerated by codon optimization according to the amino acid sequence using the GenSmart™ Codon Optimization tool (<https://www.genscript.com.cn/tools/gensmart-codon-optimization>). The translation stop codon is in purple. The ligase sequences of the *NdeI* restriction site at 5'-terminal and the *HindIII* restriction site at 3'-terminal are in blue.

>The synthesized nucleotide sequence of the recombinant iron oxidase from *A. ferriidurans* Riv11
CATATGCATCACCACCACCACCATGGAACAGTATCTAAGGCGAGCGTCCAGTACCAGGATCATC
CGCATGGTAAGCAAACTGCTCCGTGTGCGCGAACTTCGTGGCTCCGCACAGCTGCAAAGTTGT
TGCCGGCACCATCGACCCGAATGGTTGGTGTGTTGCGTTTGCGCCAAAAAGCGCATAAAGCTT

Supplementary Table S1

Table S1. Amino acid composition and property analysis of the recombinant iron oxidase from *A. ferridurans* Riv11. The hydrophobicity values of amino acid residue are from the Kyte and Doolittle hydrophobicity values [26].The net charge values at pH 2.0 of amino acid residues are computed according to the pKa values in the initial pKa values for standard amino acids [27]. The total counts for amino acid residue counts and their buried counts and exposed counts do not include N-Terminal, C-Terminal or the [Fe₄S₄] cluster.

Amino acid residue	Count	Buried count	Exposed count	Hydrophobicity	Charge at pH 7.0	Charge at pH2.0	Total charges
Ala (A)	7	0	7	1.8	0	0	0
Arg (R)	0	0	0	-4.5	+1	+1	0
Asn (N)	2	0	2	-3.5	0	0	0
Asp (D)	2	0	2	-3.5	-1	0	0
Cys (C)	4	4	0	2.5	-1	-1	-4
Gln (Q)	3	0	3	-3.5	0	0	0
Glu (E)	0	0	0	-3.5	-1	0	0
Gly (G)	4	1	3	-0.4	0	0	0
His (H)	10	0	10	-3.2	0	+1	+10
Ile (I)	1	1	0	4.5	0	0	0
Leu (L)	0	0	0	3.8	0	0	0
Lys (K)	4	0	4	-3.9	+1	+1	+4
Met (M)	1	0	1	1.9	0	0	0
Phe (F)	2	0	2	2.8	0	0	0
Pro (P)	4	0	4	-1.6	0	0	0
Ser (S)	5	0	5	-0.8	0	0	0
Thr (T)	2	0	2	-0.7	0	0	0
Trp (W)	1	0	1	-0.9	0	0	0
Tyr (Y)	1	0	1	-1.3	0	0	0
Val (V)	7	1	6	4.2	0	0	0
N-Terminal	1	0	1		+1	+1	+1
C-Terminal	1	0	1		-1	0	0
[Fe ₄ S ₄]	1	1	0		+3	+3	+3
Total	60	7	53				+14

Supplementary Figure S1



Figure S1. The multiple sequence alignment for the wild proteins of iron oxidase and HiPIP from various sources. HiPIP: high redox potential iron sulfur protein; Iro: iron oxidase. All the sequences can be obtained from NCBI Genebank (<https://www.ncbi.nlm.nih.gov/>) and their accession codes are as follows: Iro A. ferridurans Riv11:WP_215878501;Iro A. ferrianus MG:WP_239058863; Iro A. ferrivorans SS3:AEM47577;Iro A. ferrooxidans Fe1:CAA40594;Iro A. ferriphilus Riv13:WP_215860796;HiPIP A. ferrooxidans ATCC23270:ACK79288;HiPIP R. tenuis(1ISU):P33678. The signal peptide sequece are in blue. Residues conserved in all sequences are marked with an *. Residues not conserved in all sequences but conserved in some sequences are marked with (: or (.), based on the degree of conservation. The cysteine residues ligating the [Fe₄S₄] cluster are red. The key residues for interaction with ferrous ion are in blue.

Supplementary Figure S2

1: HiPIP_R_tenuis_1ISU	100.00	35.19	37.04	40.74	42.59	42.59	42.59
2: HiPIP_A_ferrooxidans_ATCC23270	35.19	100.00	54.44	55.56	56.67	53.33	53.33
3: Iro_A_ferridurans_Riv11	37.04	54.44	100.00	68.89	73.33	74.44	74.44
4: Iro_A_ferrianus_MG	40.74	55.56	68.89	100.00	77.78	80.00	78.89
5: Iro_A_ferrivorans_SS3	42.59	56.67	73.33	77.78	100.00	86.67	85.56
6: Iro_A_ferrooxidans_Fel	42.59	53.33	74.44	80.00	86.67	100.00	98.89
7: Iro_A_ferriphilus_Riv13	42.59	53.33	74.44	78.89	85.56	98.89	100.00

Figure S2. The percent identity matrix for the sequences of iron oxidase and HiPIP from various sources. The data were created by Clustal2.1 (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) according to the multiple sequence alignment in Figure S1.

Supplementary Figure S3

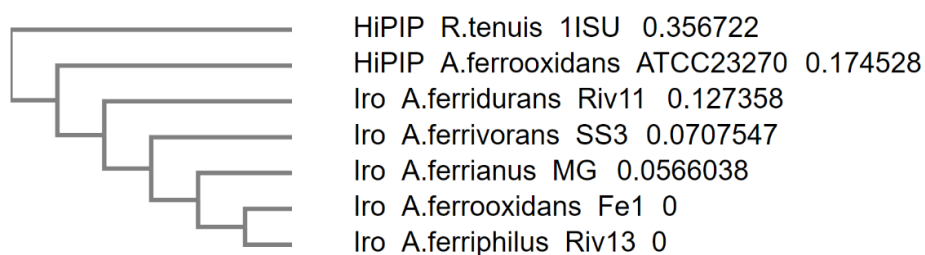


Figure S3. The phylogenetic tree for the sequences of iron oxidase and HiPIP from various sources. The data were created by Clustal2.1 (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) according to the multiple sequence alignment in Figure S1 and the percent identity matrix in Figure S2.

Supplementary Figure S4

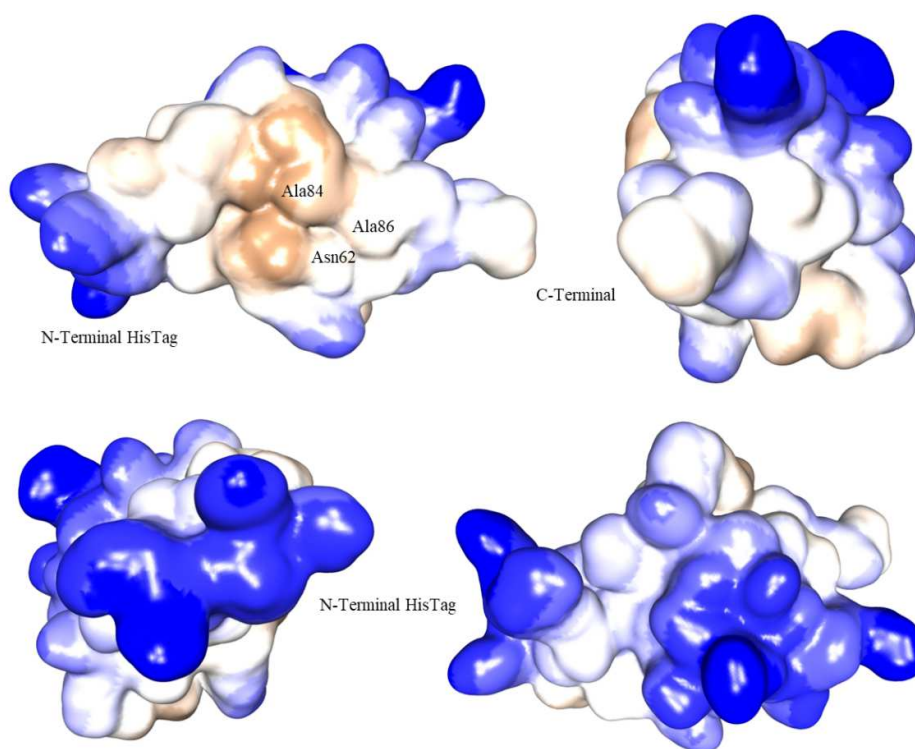


Figure S4. The hydrophobic surface analysis of iron oxidase from *A. ferridurans*. The hydrophobic property of the molecular surface was rendered according to the level, with a hydrophobicity spectrum ranging from the most hydrophobicity in brown to the most hydrophilicity in blue.

Supplementary Figure S5

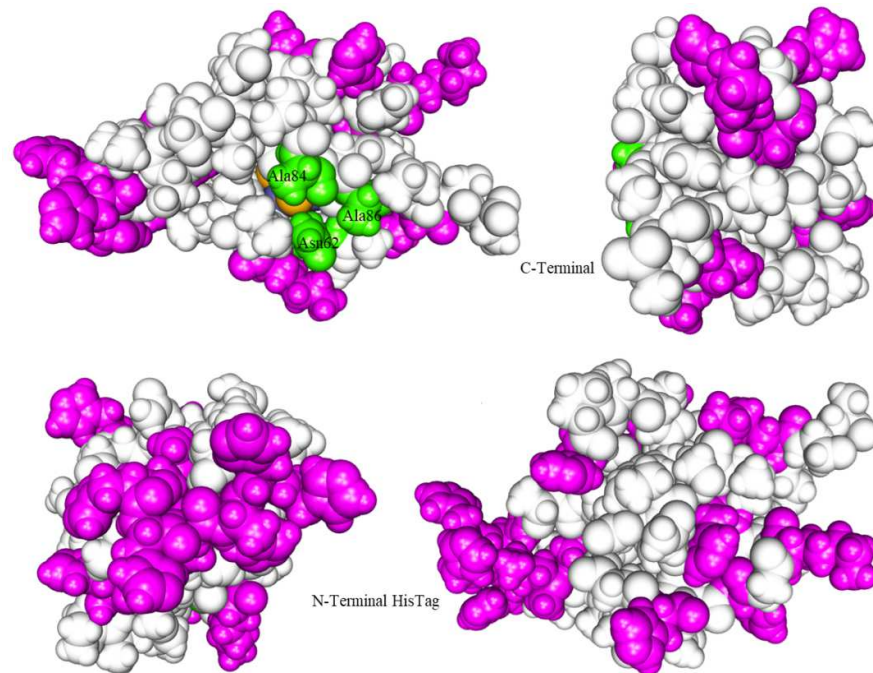


Figure S5. The surface charge analysis of recombinant iron oxidase from *A. ferriidurans*. The positively charged residues are in purple, the contact residues are in green and the other resdues in the protein are in white.