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(54) **METHODS OF PRODUCING RECOMBINANT HEME-BINDING PROTEINS AND USES THEREOF**

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(52) **U.S. Cl.** **435/25**; 435/189; 435/194; 435/252.3; 435/254.11; 435/243; 435/419; 435/348; 435/325

(57) **ABSTRACT**

The present invention is directed to methods of producing recombinant functional heme-binding proteins with complete heme incorporation and purified preparations of the same. The present invention is further directed to methods of identifying agents that modulate the activity of heme-binding proteins.

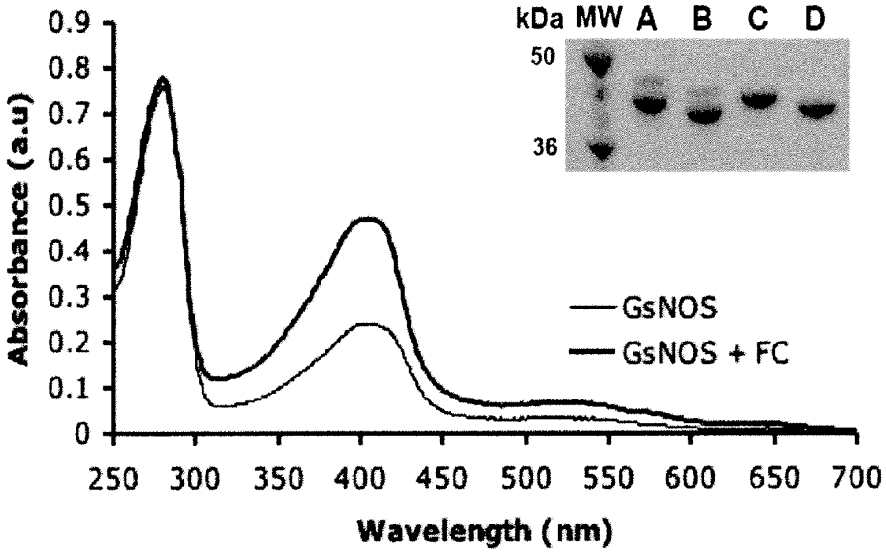
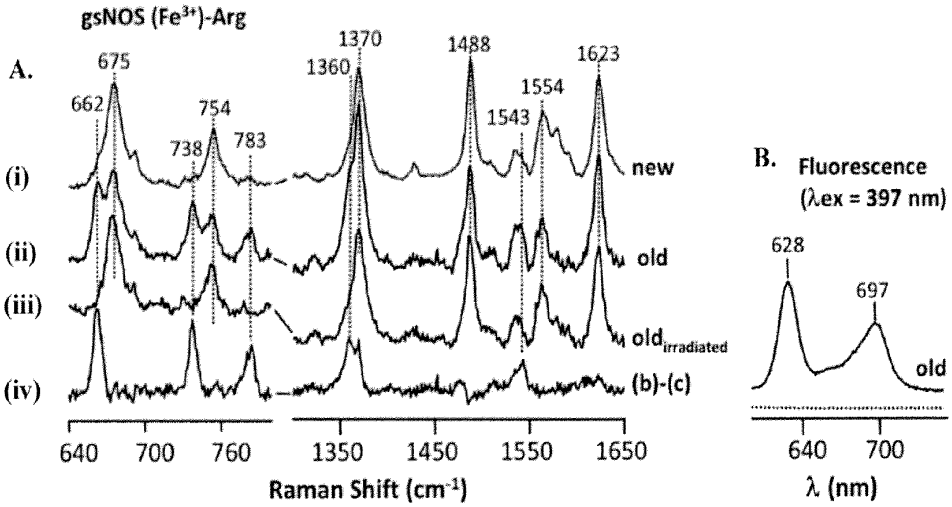

kDa MW A B C D
50
36
Absorbance (a.u)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
250 300 350 400 450 500 550 600 650 700
Wavelength (nm)
GsNOS
GsNOS + FC

Figure 1



Figures 2A–2B

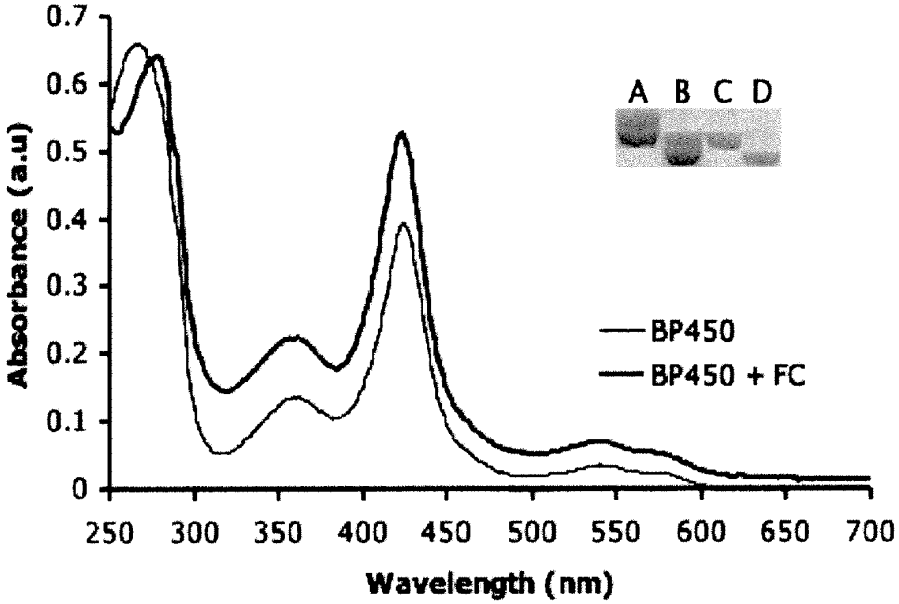

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance (a.u)
250
300
350
400
450
500
550
600
650
700
Wavelength (nm)
A B C D
BP450
BP450 + FC

Figure 3

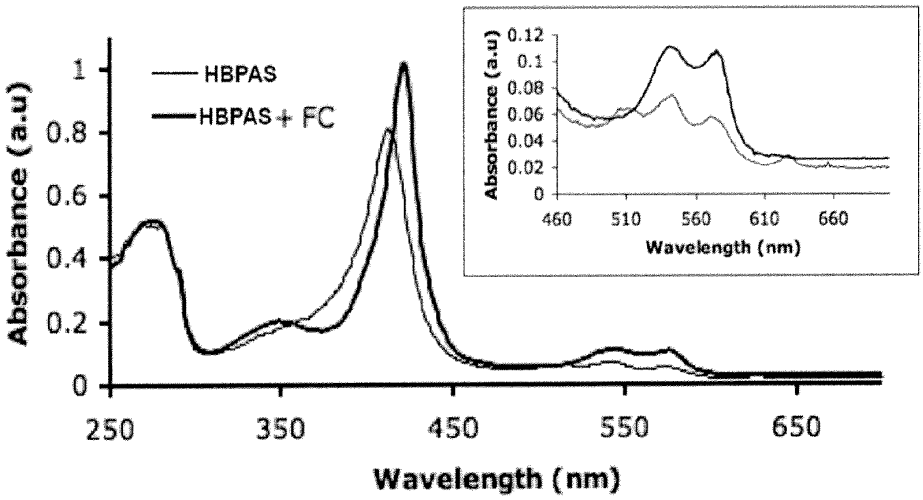

HBPAS
HBPAS + FC
Absorbance (a.u)
Wavelength (nm)
0
0.2
0.4
0.6
0.8
1
250
350
450
550
650
Absorbance (a.u)
Wavelength (nm)
0
0.02
0.04
0.06
0.08
0.1
0.12
460
510
560
610
660

Figure 4

Full-length NOS also benefits from FC-co-expresson

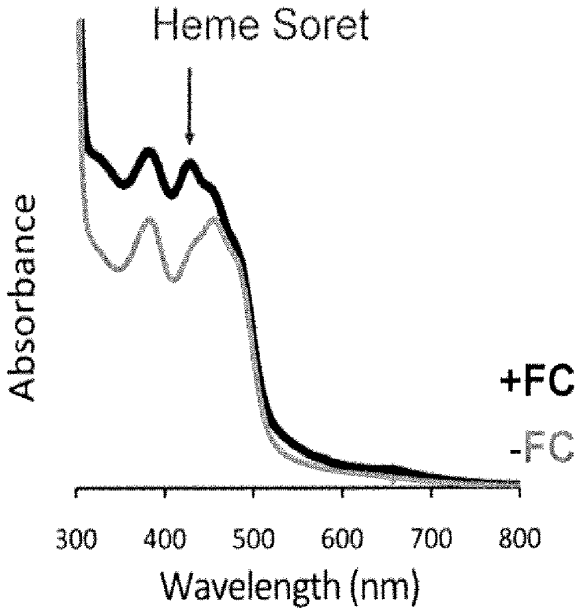


Figure 5

METHODS OF PRODUCING RECOMBINANT HEME-BINDING PROTEINS AND USES THEREOF

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. No. 61/347,193, filed May 21, 2010, which is hereby incorporated by reference in its entirety.

[0002] This invention was made with government support under grant number NCHE-0749997 awarded by the National Science Foundation and grant number R01GM079679 awarded by the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention is directed to methods of producing recombinant heme-binding proteins with complete heme incorporation and methods of using the same.

BACKGROUND OF THE INVENTION

[0004] Heme proteins encompass a wide range of functions that include electron transfer, transport and storage of oxygen, production and sensing of nitric oxide, decomposition of reactive oxygen species, catalytic oxidation of substrates, signal transduction and control of gene expression (Gray et al., "Electron Transfer in Engineered Heme Enzymes," *Faseb. J.* 11:A781-A781 (1997); Sono et al., "Heme-Containing Oxygenases," *Chem. Rev.* 96:2841-2888 (1996); Alderton et al., "Nitric Oxide Synthases Structure, Function and Inhibition," *Biochem. J.* 357:593-615 (2001); and Perutz et al., "A Haemoglobin that Acts as an Oxygen Sensor: Signalling Mechanism and Structural Basis of its Homology with PAS Domains," *Chem. Biol.* 6:R291-R297 (1999)). Under incorporation of heme into recombinant proteins can adversely influence their characterization. Evaluation of enzymatic activity is systematically underestimated if a significant proportion of the recombinant sample does not contain heme. Furthermore, proteins devoid of heme may bind another non-native cofactor with a contaminating activity of its own. A pure protein sample is usually essential for structural characterization by techniques such as X-ray crystallography, as this method often requires a single, well-folded species for crystallization. Other methods of heme protein characterization are less sensitive to the level of heme incorporation, particularly if those methods directly detect the metallocofactor (e.g., UV/Vis, EPR, and Mossbauer spectroscopy) or rely on enzymatic activity. This is both good and bad in that under incorporation may not greatly affect the analysis, but heterogeneity in the sample may go undetected.

[0005] Incomplete heme incorporation into recombinant proteins has been a frequently encountered problem (Varadarajan et al., "Cloning, Expression in *Escherichia-Coli*, and Reconstitution of Human Myoglobin," *Pro. Nat. Acad. Sci. U.S.A.* 82:5681-5684 (1985); Ishikawa et al., "Expression of Rat Heme Oxygenase in *Escherichia-Coli* as a Catalytically Active, Full-Length Form that Binds to Bacterial-Membranes," *Eur. J. Biochem.* 202:161-165 (1991); Smith et al., "Expression of a Synthetic Gene for Horseradish Peroxidase-C in *Escherichia-Coli* and Folding and Activation of the Recombinant Enzyme with Ca-2+ and Heme," *J. Biol. Chem.* 265:13335-13343 (1990); Kery et al., "Delta-Aminolevulinate Increases Heme Saturation and Yield of Human Cystathionine Beta-Synthase Expressed in *Escherichia-Coli," Archives Biochem. Biophys.* 31:624-29 (1995); Varnado et al., "Properties of a Novel Periplasmic Catalase-Peroxidase from *Escherichia Coli* O157: H7," *Archives Biochem. Biophys.* 42:1166-174 (2004); and Graves et al., "Enhancing Stability and Expression of Recombinant Human Hemoglobin in *E-Coli*: Progress in the Development of a Recombinant HBOC Source," *Biochimica Et Biophysica Acta-Proteins And Proteomics* 1784:1471-1479 (2008)) and thus, techniques have been developed to improve heme loading (Kery et al., "Delta-Aminolevulinate Increases Heme Saturation and Yield of Human Cystathionine Beta-Synthase Expressed in *Escherichia-Coli," Archives Biochem. Biophys.* 31:624'29 (1995); Varnado et al., "Properties of a Novel Periplasmic Catalase-Peroxidase from *Escherichia Coli* O157: H7," *Archives Biochem. Biophys.* 42:1166-174 (2004); Graves et al., "Enhancing Stability and Expression of Recombinant Human Hemoglobin in *E-Coli*: Progress in the Development of a Recombinant HBOC Source," *Biochimica Et Biophysica Acta-Proteins And Proteomics* 1784:1471-1479 (2008); Weickert et al., "Optimization of Heterologous Protein Production in *Escherichia Coli," Curr. Opinion In Biotechnol.* 7:494-499 (1996); Shen et al., "Production of Unmodified Human Adult Hemoglobin in *Escherichia-Coli," Pro. Nat, Acad. Sci. U.S.A.* 90:8108-8112 (1993); Varnado et al., "System for the Expression of Recombinant Hemoproteins in *Escherichia Coli," Prot. Exp. Pur.* 35:76-83 (2004); and Vaniado et al., "Expression of Recombinant Hemoproteins in *E. Coli* Using a Heme Protein Expression System," *Biophys. J.* 384A-384A (2007)). During induction of recombinant protein expression from highly active vectors, such as those that employ the T7-polymerase, a population of protein will fold without the heme co-factor under conditions where folding outpaces heme delivery (Weickert et al., "Blackmore, Stabilization of Apoglobin by Low Temperature Increases Yield of Soluble Recombinant Hemoglobin in *Escherichia Coli," App. Environ. Microbiol.* 63:4313-4320 (1997)). Supplementing the growth media with δ-amino levulinic acid (d-ALA), a precursor in the C5 heme biosynthesis pathway, increases levels of heme biosynthesis and thereby heme incorporation into the target protein (Kery et al., "Delta-Aminolevulinate Increases Heme Saturation and Yield of Human Cystathionine Beta-Synthase Expressed in *Escherichia-Coli," Archives Biochem. Biophys.* 31:624-29 (1995); Pcsce et al., "The 109 Residue Nerve Tissue Minihemoglobin from Cerebratulus Lacteus Highlights Striking Structural Plasticity of the Alpha-Helical Globin Fold," *Structure* 10:725-735 (2002); and Summerford et al., "Bacterial Expression of Scapharca Dimeric Hemoglobin—A Simple-Model System for Investigating Protein Cooperativity," *Prot. Engineer.* 8:593-599 (1995)). Increased heme biosynthesis rates through d-ALA supplementation does not achieve complete heme incorporation into all heme-binding proteins (Weickert et al., "Optimization of Heterologous Protein Production in *Escherichia Coli," Curr. Opinion In Biotechnol.* 7:494-499 (1996); Shen et al., "Production of Unmodified Human Adult Hemoglobin in *Escherichia-Coli," Pro. Nat. Acad. Sci. U.S.A.* 90:8108-8112 (1993); Weickert et al., "High-Fidelity Translation of Recombinant Human Hemoglobin in *Escherichia Coli," Appl. Environ. Microbiol.* 64:589-1593 (1998); and Wcickert et al., "A Mutation that Improves Soluble Recombinant Hemoglobin Accumulation in *Escherichia Coli* in Heme Excess," *App. Environ. Microbiol.* 65:640-647 (1999)).

[0006] Another technique for increasing home incorporation into recombinant proteins involves supplying the bacteria with hemin in the growth media. However, most *E. coli* strains do not possess an efficient heme transport system, and thus uptake of hemin relies on diffusion through the cell membrane. As a result, hemin feeding is much more effective with strains that co-express heme transport genes from other gram-negative bacteria, along with the heme-protein of interest (Graves et al., "Enhancing Stability and Expression of Recombinant Human Hemoglobin in *E-Coli*: Progress in the Development of a Recombinant HBOC Source," *Biochimica Et Biophysica Acta—Proteins And Proteomics* 1784:1471-1479 (2008); Varnado et al., "System for the Expression of Recombinant Hemoproteins in *Escherichia Coli," Prot. Exp. Pur.* 35:76-83 (2004); and Varnado et al., "Expression of Recombinant Hemoproteins in *E. Coli* Using a Heme Protein Expression System," *Biophys. J.* 384A-384A (2007)). For example, co-expression of the heme transport system from *P. shigelloides*, which consists of the proteins Hug A/B/C/D, TonB, and Exb B/D, while also supplementing the growth media with hemin, results in higher amounts of the target holo-protein (in this case hemoglobin) (Graves et al., "Enhancing Stability and Expression of Recombinant Human Hemoglobin in *E-Coli*: Progress in the Development of a Recombinant HBOC Source," *Biochimica Et Biophysica Acta—Proteins And Proteomics* 1784:1471-1479 (2008)). A similar method involves the co-expression of the heme receptor ChuA from *E. coli*. strain O157:H7 to enhance hemin (Varnado et al., "System for the Expression of Recombinant Hemoproteins in *Escherichia Coli," Prot. Exp. Pur.* 35:76-83 (2004)). This latter method also shows a significant increase in the amount of heme-loaded protein generated, although in both cases, the ratio of holoprotein:apoprotein was not evaluated. Another approach utilizes the heme-permeability of *E. coli* strain RP523, which has the hem B, porphobilinogen synthase gene disrupted to prevent native heme synthesis. All heme and/or heme analogs are procured by the cells from the growth media and incorporation is nearly stoichiometric for proteins expressed in the cytoplasm (0.8-1.0 heme/heme analog per protein) (Woodward et al., "An *Escherichia Coli* Expression-Based Method for Heme Substitution," *Nat. Methods* 4:43-45 (2007)).

[0007] Full incorporation of heme in recombinant proteins is also important for commercial applications. For example, the feasibility of employing recombinant human hemoglobin as an oxygen delivery pharmaceutical is limited by the yield of holoprotein that can be made in *E. coli* (Graves et al. "Enhancing Stability and Expression of Recombinant Human Hemoglobin in *E-Coli*: Progress in the Development of a Recombinant HBOC Source," *Biochimica Et Biophysica Acta—Proteins And Proteomics* 1784:1471-1479 (2008)). Some of the methods discussed above, while effective, require co-expression of several heme transport proteins, which could limit yields, and/or require addition of the heme cofactor.

[0008] The present invention is directed to overcoming these and other deficiencies in the art.

SUMMARY OF THE INVENTION

[0009] A first aspect of the present invention relates to a method of producing a functional recombinant heme-binding protein. This method involves co-expressing a recombinant heme-binding protein and recombinant ferrochelatase protein, or polypeptide thereof, under conditions effective for complete heme incorporation into the recombinantly produced heme-binding protein, thereby producing a functional heme-binding protein.

[0010] A second aspect of the present invention relates to a system for producing functional heme-binding proteins. This system comprises an expression system and one or more expression constructs encoding a recombinant heme-binding protein and a recombinant ferrochelatase.

[0011] A third aspect of the present invention relates to a purified preparation of recombinant functional heme-binding protein.

[0012] A fourth aspect of the present invention relates to a method of identifying an agent that modulates activity of a heme-binding protein. This method involves providing a candidate agent and providing a recombinant functional heme-binding protein. This method further involves contacting the candidate agent with the recombinant functional heme-binding protein under conditions at which the functional heme-binding protein is active and comparing the activity of the functional heme-binding protein as a result of said contacting to the activity of the heme-binding protein alone, both under said conditions at which the heme-binding protein is active. A candidate agent that modulates the activity of a heme-binding protein is identified based on said comparing.

[0013] Another aspect of the present invention relates to a method of evaluating the metabolism of an agent by a heme-binding protein. This method involves providing a candidate agent and providing a recombinant functional heme-binding protein. This method further involves contacting the candidate agent with the recombinant functional home-binding protein under conditions at which the functional heme-binding protein is active and comparing the activity of the functional heme-binding protein as a result of said contacting to the activity of the heme-binding protein alone, both under said conditions at which the heme-binding protein is active. The metabolism of the candidate agent by a heme-binding protein is evaluated based on said comparing.

[0014] The present invention is a straightforward and inexpensive method for high fidelity incorporation of heme into recombinantly overexpressed heme proteins. Co-expression of just one native protein, ferrochelatase (FC), in the presence of δ-ALA is sufficient to achieve 100% heme incorporation into three unrelated home proteins derived from different organisms. Since pre-existing methods of recombinant heme-binding protein production result in sub-optimal heme incorporation and varying amounts of protein production depending on the protein of interest, the ability to achieve complete heme incorporation as described herein, has important implications for heme-binding protein biochemical characterization, spectroscopy, structural studies, and for the production of homogeneous commercial heme-binding proteins with high activity.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. **1** shows the UV-Vis spectra of *Geobacillus stearothermophilus* nitric oxide synthase (gsNOS) expressed alone (thin line) and gsNOS expressed with ferrochelatase (FC) (thick line). Co-expression of FC results in a substantial increase in home content of gsNOS as measured by the Abs_{403}/Abs_{280} ratio. This value saturates at 0.6, which indicates nearly complete heme incorporation. The inset of FIG. **1** is an immunoblot showing that GsNOS expressed by itself results in two bands on SDS-PAGE (lane A), both of which shift on His-tag cleavage (lane B), GsNOS co-expressed with

FC results in just one band (lane C), that shifts on His-tag cleavage (lane D) as expected.

[0016] FIGS. **2**A-**2**B are Resonance Raman and fluorescence spectra of recombinantly expressed gsNOS. The Resonance Raman spectra from gsNOS co-expressed with FC is shown as FIG. **2**A(i), and gsNOS expressed by itself is depicted in FIGS. **2**A(ii) and **2**A(iii). The spectrum in FIG. **2**A(ii) is obtained with 3 mW of 413.1 nm laser excitation with an acquisition time of 5 min. The spectrum in FIG. **2**A(iii) is from the same sample as FIG. **2**A(ii), but obtained after prolonged irradiation with 42 mW of 413.1 nm laser for two hours. FIG. **2**A(iv) shows the difference spectrum (i.e., the spectrum of FIG. **2**A(ii)—the spectrum of FIG. **2**A(iii)), which highlights spectral lines resulting from contamination by photosensitive protoporphyrin IX, The fluorescence spectrum shown in FIG. **2**B obtained after a 397 nm excitation is representative of that of protoporphyrin IX bound to protein (see infra), and is from the same sample as FIG. **2**B.

[0017] FIG. **3** shows the UV-Vis spectra of BP450 expressed by itself (thin line) and BP450 expressed with FC (thick line). Co-expression of FC results in a substantial increase in heme content of BP450 (Abs_{Soret}/Abs_{280}). The inset of FIG. **3** is an immunoblot showing that BP450 expressed alone also results in two bands (lane A), both of which shift on His-tag cleavage (lane B). BP450 co-expressed with FC results in one band (lane C), which shifts on His-tag cleavage as expected (lane D).

[0018] FIG. **4** shows the UV-Vis spectra of a Heme Binding PAS domain (HBPAS; His-ligated). When expressed by itself (thin line), the UV-Vis spectra of HBPAS shows four Q-hand absorption peaks (FIG. **4** inset). Co-expression with FC (thick line) increases the heme content and results in only two Q-bands (inset).

[0019] FIG. **5** shows the UV-Vis spectra of full-length NOS containing a reductase domain (>100 KdA). Under normal conditions of expression the Soret band indicative of heme incorporation is barely visible above the flavin absorption of the reductase component (gray trace), however, upon co-expression with ferrochelatase a strong Soret absorption peak (at ~416 nm), indicative of heme incorporation becomes apparent (black trace), Total yields of full-length NOS also increase in the presence of ferrochelatase.

DETAILED DESCRIPTION OF THE INVENTION

[0020] A first aspect of the present invention relates to a method of producing functional recombinant heme-binding proteins. This method involves co-expressing a recombinant heme-binding protein and recombinant ferrochelatase protein, or polypeptide thereof, under conditions effective for complete heme incorporation into the recombinantly produced heme-binding protein, thereby producing a functional heme-binding protein.

[0021] In accordance with this aspect of the invention, a heme-binding protein encompasses any protein that contains a heme prosthetic group either covalently or noncovalently bound to itself. Heme-binding proteins have diverse biological functions including, oxygen transport, catalysis, active membrane transport, electron transport, and sensory. The various classes of heme-binding proteins that are encompassed by the methods of the present invention include, without limitation, globins (e.g., hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin), cytochromes (e.g., a-, b-, and c-types, cd1-nitrite reductase, cytochrome oxidase), transferrins (e.g., lactotransferrin, serotransferrin, melanotransferrin), bacterioferririns, hydroxylamine oxidoreductase, nitrophorins, peroxidases (e.g., lignin peroxidase), cyclooxygenases (e.g., COX-1, COX-2, COX-3, prostaglandin H synthase), catalases, cytochrome P-450s, chloroperoxidases, PAS-domain heme sensors, H-NOX heme sensors (e.g., soluble guanylate cyclase, FixL, DOS, HemAT, and CooA), heme-oxygenases, and nitric oxide synthases. The recombinant heme-binding protein produced using the methods of the present invention can be prokaryotic or eukaryotic. For example, in one embodiment of the present invention the recombinant heme-binding protein is mammalian, preferably human. In another embodiment of the present invention the recombinant heme-binding protein is bacterial. In yet another embodiment of the present invention, the recombinant heme-binding protein is fungal, preferably yeast.

[0022] Table 1 provides a non-exhaustive list of exemplary human heme-binding proteins that are suitable for production using the methods of the present invention. Table 1 identifies each heme-binding protein by its Universal Protein Resource Knowledgebase (UniProtKB)/Swiss Prot accession number, which provides the amino acid sequence of the identified protein, and the EMBL Nucleotide Database accession number, which provides the nucleotide sequence encoding the heme-binding protein. The UniProtKB/Swiss Prot and EMBL accession numbers, along with the corresponding amino acid and nucleotide sequence information for each entry in Table 1 is hereby incorporated by reference. Table 1 further identifies the UniProtKB/Swiss Prot entry name, protein names and gene names for each identified heme-binding protein.

[0023] Table 2 provides a list of lignin peroxidases, which are heme-binding proteins also suitable for production using the methods of the present invention. As described herein, lignin peroxidases metabolism the lignin of plant cell walls which facilitates the breakdown of cell wall polysaccharides to simple sugars and the subsequent conversion of these sugars to usable bio-fuel. Table 2 identifies each lignin peroxidase by its UniProtKB/Swiss Prot accession number, which provides the amino acid sequence of the protein, and the EMBL Nucleotide Database accession number, which provides the encoding nucleotide sequence. The UniProtKB/Swiss Prot and EMBL accession numbers, along with the corresponding amino acid and nucleotide sequence information for each entry in Table 2 is hereby incorporated by reference. Table 2 further identifies the UniProtKB/Swiss Prot entry name, protein names, protein family, gene names, and organism for each entry.

[0024] Traditional methods of producing recombinant heme-binding proteins generate recombinant proteins having incomplete heme incorporation. Under incorporation of heme into recombinant proteins can adversely influence their function. In contrast to these traditional methods, the methods of the present invention generate recombinant heme-binding proteins that have complete heme incorporation. Therefore, these proteins are completely functional and are more suitable for research, clinical, and commercial applications. Incomplete heme incorporation into a recombinant protein can be detected by the presence of free base porphyrin using fluorescence spectroscope

TABLE 1

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
Globins	Q8WWM9	CYGB_HUMAN	Cytoglobin (Histoglobin) (HGb) (Stellate cell activation-associated protein)	CYGB STAP	AJ315162; AB057769; AK098057; CH471099; BC029798;
	P09105	HBAT_HUMAN	Hemoglobin subunit theta-1 (Hemoglobin theta-1 chain) (Theta-1-globin)	HBQ1	X06482; M33022; DQ431198; AE006462; BC056686; M91453;
	P02008	HBAZ_HUMAN	Hemoglobin subunit zeta (HBAZ) (Hemoglobin zeta chain) (Zeta-globin)	HBZ HBZ2	J00182; M24173; Z84721; CR456848; AE006462; CH471112; BC027892;
	P69905	HBA_HUMAN	Hemoglobin subunit alpha (Alpha-globin) (Hemoglobin alpha chain)	HBA1; HBA2	J00153; J00153; V00491; V00493; V00488; V00516; AF230076; AF525460; DQ431198; DQ431198; AF097635; AF105974; AF349571; AF536204; DQ499017; DQ499018; AK223392; AE006462; AE006462; Z84721; Z84721; BC005931; BC008572; BC032122; BC050661; BC101846; BC101848;
	P68871	HBB_HUMAN	Hemoglobin subunit beta (Beta-globin) (Hemoglobin beta chain) [Cleaved into: LVV-hemorphin-7]	HBB	M25079; V00499; DQ126270; DQ126271; DQ126272; DQ126273; DQ126274; DQ126275; DQ126276; DQ126277; DQ126278; DQ126279; DQ126280; DQ126281; DQ126282; DQ126283; DQ126284; DQ126285; DQ126286; DQ126287; DQ126288; DQ126289; DQ126290; DQ126291; DQ126292; DQ126293; DQ126294; DQ126295; DQ126296; DQ126297; DQ126298; DQ126299; DQ126300; DQ126301; DQ126302; DQ126303; DQ126304; DQ126305; DQ126306; DQ126307; DQ126308; DQ126309; DQ126310; DQ126311; DQ126312; DQ126313; DQ126314; DQ126315; DQ126316; DQ126317; DQ126318; DQ126319; DQ126320; DQ126321; DQ126322; DQ126323; DQ126324; DQ126325; AF007546; AF083883; AF117710; AF181989; AF349114; AF527577; AY136510; AY163866; AY260740; AY509193; EF450778; EU694432; AK311825; CR536530; CR541913; CH471064; BC007075; U01317; V00497; V00500; L26462; L26463; L26464; L26465; L26466; L26467; L26468; L26469; L26470; L26471; L26472; L26473; L26
	P02042	HBD_HUMAN	Hemoglobin subunit delta (Delta-globin) (Hemoglobin delta chain)	HBD	U01317; V00505; AF339401; AF339402; AF339403; AF339404; AF339405; AF339406; AF339407; AF339408; AF339409; AF339410; AF339411; AF339412; AF339413; AF339414; AF339415; AF339416; AF339417; AY034468; DQ157442; BC069307; BC070282;
	P02100	HBE_HUMAN	Hemoglobin subunit epsilon (Epsilon-globin) (Hemoglobin epsilon chain)	HBE1 HBE	U01317; V00508; CR541912; CH471064; BC015537;
	P69891	HBG1_HUMAN	Hemoglobin subunit gamma-1 (Gamma-1-globin) (Hb F Agamma) (Hemoglobin gamma-1 chain) (Hemoglobin gamma-A chain)	HBG1 PRO2979	M91036; M91037; V00513; V00514; J00176; U01317; AF130098; CH471064; BC010913; BC020719; AF487523;
	P69892	HBG2_HUMAN	Hemoglobin subunit gamma-2 (Gamma-2-globin) (Hb F Ggamma) (Hemoglobin gamma-2 chain)	HBG2	M91036; M91037; U01317; V00515; M15386; AY662983; AK290492; BC010914; BC029387; BC130457; BC130459; M11427;

TABLE 1-continued

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
			(Hemoglobin gamma-G chain)		
	Q6B0K9	HBM_HUMAN	Hemoglobin subunit mu (Hemoglobin mu chain) (Mu-globin)	HBM HBAP2	AY698022; DQ431198; BC035682;
	P02144	MYG_HUMAN	Myoglobin	MB	X00371; X00372; X00373; M14603; M10090; M14602; CR456516; CR541949; DQ003030; AL022334; AL049747; BC014547;
	Q9NPG2	NGB_HUMAN	Neuroglobin	NGB	AJ245944; AJ245946; AF422796; AF422797; AC007375; AC007954; BC032509;
Cyto-chrome P450s	P15538	C11B1_HUMAN	Cytochrome P450 11B1, mitochondrial (CYPXIB1) (Cytochrome P-450c11) (Cytochrome P450C11) (Steroid 11-beta-hydroxylase) (EC 1.14.15.4)	CYP11B1 S11BH	M32879; M32863; M32878; X55764; D16153; D16155; EU332839; CH471162; BC096287; M24667; D10169;
	P19099	C11B2_HUMAN	Cytochrome P450 11B2, mitochondrial (Aldosterone synthase) (ALDOS) (EC 1.14.15.4) (EC 1.14.15.5) (Aldosterone-synthesizing enzyme) (CYPXIB2) (Cytochrome P-450Aldo) (Cytochrome P-450C18) (Steroid 18-hydroxylase)	CYP11B2	M32881; M32864; M32880; X54741; D13752; EU326306; CH471162;
	A6NCC7	C21AL_HUMAN	Putative cytochrome P450 21-like protein ENSP00000364438		
	Q4G0S4	C27C1_HUMAN	Cytochrome P450 27C1 (EC 1.14.—.—)	CYP27C1	AK131190; BC039307;
	Q6ZSU1	C2G1L_HUMAN	Putative cytochrome P450 2G1-like protein		AK127151;
	P05108	CP11A_HUMAN	Cholesterol side-chain cleavage enzyme, mitochondrial (EC 1.14.15.6) (CYPXIA1) (Cholesterol desmolase) (Cytochrome P450 11A1) (Cytochrome P450(scc))	CYP11A1 CYP11A	M14565; X05367; X05368; X05369; X05370; X05371; X05372; X05373; X05374; AK292300; CH471136; BC032329; X14257; M28253;
	P05093	CP17A_HUMAN	Steroid 17-alpha-hydroxylase/17,20 lyase (EC 1.14.99.9) (CYPXVII) (Cytochrome P450 17A1) (Cytochrome P450-C17) (Cytochrome P450c17) (Steroid 17-alpha-monooxygenase)	CYP17A1 CYP17 S17AH	M14564; M19489; M63871; M31153; M31146; M31147; M31148; M31149; M31150; M31151; M31152; BT020000; AL358790; BC062997; BC063388;
	P11511	CP19A_HUMAN	Cytochrome P450 19A1 (EC 1.14.14.1) (Aromatase) (CYPXIX) (Cytochrome P-450AROM) (Estrogen synthase)	CYP19A1 ARO1 CYAR CYP19	M22246; X13589; M18856; J04127; Y07508; M30804; M30796; M30797; M30798; M30800; M30801; M30802; M30803; AY957953; BC107785; M28420;
	P04798	CP1A1_HUMAN	Cytochrome P450 1A1 (EC 1.14.14.1) (CYPIA1) (Cytochrome P450 form 6) (Cytochrome P450-C) (Cytochrome P450-P1)	CYP1A1	X02612; K03191; X04300; AF253322; AK223113; BC023019; M12079; AF040259;
	P05177	CP1A2_HUMAN	Cytochrome P450 1A2 (EC 1.14.14.1) (CYPIA2) (Cytochrome P(3)450) (Cytochrome P450 4) (Cytochrome P450-P3)	CYP1A2	Z00036; L00389; L00384; L00385; L00386; L00388; L00387; M31667; M31664; M31665; M31666; M12078; AF182274; AF253322; DQ022432; BC067424; BC067425; BC067426; BC067427; BC067428; M55053;

TABLE 1-continued

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
	Q16678	CP1B1_HUMAN	Cytochrome P450 1B1 (EC 1.14.14.1) (CYPIB1)	CYP1B1	U03688; U56438; AF450132; AF450131; BT019979; AY393998; BC012049; AF171066;
	Q6UW02	CP20A_HUMAN	Cytochrome P450 20A1 (EC 1.14.—.—)	CYP20A1 UNQ667/PRO1301	AY359068; AC011737; BC020616; BC033752;
	P08686	CP21A_HUMAN	Steroid 21-hydroxylase (EC 1.14.99.10) (21-OHase) (Cytochrome P-450c21) (Cytochrome P450 21) (Cytochrome P450 XXI) (Cytochrome P450-C21) (Cytochrome P450-C21B)	CYP21A2 CYP21 CYP21B	M12792; M13936; M26856; X58906; BC125182; K02771; M19711; M17252;
	Q07973	CP24A_HUMAN	1,25-dihydroxyvitamin D(3) 24-hydroxylase, mitochondrial (24-OHase) (Vitamin D(3) 24-hydroxylase) (EC 1.14.13.n4) (Cytochrome P450 24A1) (Cytochrome P450-CC24)	CYP24A1 CYP24	L13286; AL138805; U60669; S67623;
	O43174	CP26A_HUMAN	Cytochrome P450 26A1 (EC 1.14.—.—) (Cytochrome P450 retinoic acid-inactivating 1) (Cytochrome P450RAI) (hP450RAI) (Retinoic acid 4-hydroxylase) (Retinoic acid-metabolizing cytochrome)	CYP26A1 CYP26 P450RAI1	AF005418; AL358613; CH471066;
	Q9NR63	CP26B_HUMAN	Cytochrome P450 26B1 (EC 1.14.—.—) (Cytochrome P450 26A2) (Cytochrome P450 retinoic acid-inactivating 2) (Cytochrome P450RAI-2) (Retinoic acid-metabolizing cytochrome)	CYP26B1 CYP26A2 P450RAI2	AF252297; AC007002; AK313433; BC069443; BC109205;
	Q8V0L0	CP26C_HUMAN	Cytochrome P450 26C1 (EC 1.14.—.—)	CYP26C1	AY356349; AL358613;
	Q02318	CP27A_HUMAN	Sterol 26-hydroxylase, mitochondrial (EC 1.14.13.15) (5-beta-cholestane-3-alpha,7-alpha,12-alpha-triol 27-hydroxylase) (Cytochrome P-450C27/25) (Cytochrome P450 27) (Sterol 27-hydroxylase) (Vitamin D(3) 25-hydroxylase)	CYP27A1 CYP27	M62401; X59812; AY178622; AK290418; CH471063; BC040430; BC051851; S62709;
	O15528	CP27B_HUMAN	25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial (EC 1.14.13.13) (25-OHD-1 alpha-hydroxylase) (25-hydroxyvitamin D(3) 1-alpha-hydroxylase) (VD3 1A hydroxylase) (Calcidiol 1-monooxygenase) (Cytochrome P450 subfamily XXVIIB polypeptide 1) (Cytochrome P450C1 alpha) (Cytochrome P450VD1-alpha) (Cytochrome p450 27B1)	CYP27B1 CYP1ALPHA CYP27B	AF027152; AB005038; AB005989; AB005990; AB006987; AF020192; AF246895; AY288916;
	P11509	CP2A6_HUMAN	Cytochrome P450 2A6 (EC 1.14.14.1) (CYPIIA6) (Coumarin 7-hydroxylase) (Cytochrome P450 IIA3) (Cytochrome P45D(I))	CYP2A6 CYP2A3	X13897; X13929; X13930; M33318; AF182275; AK312964; EU135979; FJ440681; CH471126; BC096253; BC096254; BC096255; BC096256; AF326721; K03192;

TABLE 1-continued

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
	P20853	CP2A7_HUMAN	Cytochrome P450 2A7 (EC 1.14.14.1) (CYPIIA7) (Cytochrome P450 IIA4)	CYP2A7	M33317; U22029;
	Q16696	CP2AD_HUMAN	Cytochrome P450 2A13 (EC 1.14.14.1) (CYPIIA13)	CYP2A13	U22028; AF209774; AY513604; AY513605; AY513606; AY513608; AY513609;
	P20813	CP2B6_HUMAN	Cytochrome P450 2B6 (EC 1.14.14.1) (CYPIIB6) (Cytochrome P450 IIB1)	CYP2B6	M29874; AF182277; DQ298753; AC023172;
	P10632	CP2C8_HUMAN	Cytochrome P450 2C8 (EC 1.14.14.1) (CYPIIC8) (Cytochrome P450 IIC2) (Cytochrome P450 MP-12) (Cytochrome P450 MP-20) (Cytochrome P450 form 1) (S-mephenytoin 4-hydroxylase)	CYP2C8	M17397; M17398; Y00498; AK292753; AK315823; AY514490; AL359672; CH471066; BC020596; X54807; M21941; M21942; X51535;
	P11712	CP2C9_HUMAN	Cytochrome P450 2C9 ((R)-limonene 6-monooxygenase) (EC 1.14.13.80) ((S)-limonene 6-monooxygenase) (EC 1.14.13.48) ((S)-limonene 7-monooxygenase) (EC 1.14.13.49) (CYPIIC9) (Cytochrome P-450MP) (Cytochrome P450 MP-4) (Cytochrome P450 MP-8) (Cytochrome P450 PB-1) (S-mephenytoin 4-hydroxylase)	CYP2C9 CYP2C10	AY341248; AY702706; D00173; M15331; M21939; M21940; S46963;
	P33260	CP2CI_HUMAN	Cytochrome P450 2C18 (EC 1.14.14.1) (CYPIIC18) (Cytochrome P450-6b/29c)	CYP2C18	M61856; L16876; L16871; L16872; L16869; L16870; L16875; L16873; L16874; AK313403; AL583836; CH471066; BC069666; BC096257; BC096258;
	P33261	CP2CJ_HUMAN	Cytochrome P450 2C19 ((R)-limonene 6-monooxygenase) (EC 1.14.13.80) ((S)-limonene 6-monooxygenase) (EC 1.14.13.48) ((S)-limonene 7-monooxygenase) (EC 1.14.13.49) (CYPIIC17) (CYPIIC19) (Cytochrome P450-11A) (Cytochrome P450-254C) (Mephenytoin 4-hydroxylase)	CYP2C19	M61854; M61858; L07093; AY796203; AL583836; AL133513; AL133513; AL583836; L39098; L39097; L39102; L39099; L39100; L39101;
	P10635	CP2D6_HUMAN	Cytochrome P450 2D6 (EC 1.14.14.1) (CYPIID6) (Cytochrome P450-DB1) (Debrisoquine 4-hydroxylase)	CYP2D6 CYP2DL1	M20403; X08006; M33388; AY545216; DQ282144; DQ282145; DQ282146; DQ282151; DQ282154; DQ282155; BC075023; BC075024;
	P05181	CP2E1_HUMAN	Cytochrome P450 2E1 (EC 1.14.13.—) (4-nitrophenol 2-hydroxylase) (EC 1.14.13.n7) (CYPIIE1) (Cytochrome P450-J)	CYP2E1 CYP2E	J02625; J02843; AF182276; DQ515958; AL161645; CH471211; AF084225; D50111;
	P24903	CP2F1_HUMAN	Cytochrome P450 2F1 (EC 1.14.14.1) (CYPIIF1)	CYP2F1	J02906; AF372573; AF372570; AF372571; AF372572; EF122241; EF122242; EF122243; EF122244; EF122245;
	P51589	CP2J2_HUMAN	Cytochrome P450 2J2 (EC 1.14.14.1) (Arachidonic acid epoxygenase) (CYPIIJ2)	CYP2J2	U37143; AF272142; AY426985; BC032594;
	Q6VVX0	CP2R1_HUMAN	Vitamin D 25-hydroxylase (EC 1.14.13.15) (Cytochrome P450 2R1)	CYP2R1	AY323817; BC104907; BC104909; AY800276;
	Q96SQ9	CP2S1_HUMAN	Cytochrome P450 2S1 (EC 1.14.14.1) (CYPIIS1)	CYP2S1 UNQ891/PRO1906	AF335278; AY358603; AK027605; BC033691;
	Q7Z449	CP2U1_HUMAN	Cytochrome P450 2U1 (EC 1.14.14.1)	CYP2U1	AY343323; CH471057; BC012027; BC132767; BC136483;

TABLE 1-continued

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
	Q8TAV3	CP2W1_HUMAN	Cytochrome P450 2W1 (EC 1.14.14.—) (CYPIIW1)	CYP2W1	BC025761;
	Q9HB55	CP343_HUMAN	Cytochrome P450 3A43 (EC 1.14.14.1)	CYP3A43	AF319634; AF337813; AF280107; AF280108; AF280109; AF280110; AF280111; AY390423; AY390424; AY390425; AY390426;
	Q9NYL5	CP39A_HUMAN	24-hydroxycholesterol 7-alpha-hydroxylase (Oxysterol 7-alpha-hydroxylase) (EC 1.14.13.99) (Cytochrome P450 39A1) (hCYP39A1)	CYP39A1	AF237982; AK292283; AL591242; AL035670; AL035670; AL591242; BC010358;
	P08684	CP3A4_HUMAN	Cytochrome P450 3A4 (Albendazole monooxygenase) (EC 1.14.13.32) (Albendazole sulfoxidase) (CYPIIIA3) (CYPIIIA4) (Cytochrome P450 3A3) (Cytochrome P45C HLp) (Cytochrome P450 NF-25) (Cytochrome P450-PCN1) (Nifedipine oxidase) (Quinine 3-monooxygenase) (EC 1.14.13.67) (Taurochenodeoxycholate 6-alpha-hydroxylase) (EC 1.14.13.97)	CYP3A4 CYP3A3	D00003; M13785; M18907; M14096; X12387; J04449; AF182273; AF280107; AF209389;
	P20815	CP3A5_HUMAN	Cytochrome P450 3A5 (EC 1.14.14.1) (CYPIIIA5) (Cytochrome P450 HLp2) (Cytochrome P450-PCN3)	CYP3A5	J04813; AC005020; CH236956; CH471091; BC033862; AF280107; L35912; S74699; S74700;
	P24462	CP3A7_HUMAN	Cytochrome P450 3A7 (EC 1.14.14.1) (CYPIIIA7) (Cytochrome P450-HFLA)	CYP3A7	D00408; AF280107; CH236956; CH471091; BC067436;
	Q9Y6A2	CP46A_HUMAN	Cholesterol 24-hydroxylase (CH24H) (EC 1.14.13.98) (Cytochrome P450 46A1)	CYP46A1 CYP46	AF094480; BC022539;
	Q02928	CP4AB_HUMAN	Cytochrome P450 4A11 (20-hydroxyeicosatetraenoic acid synthase) (20-HETE synthase) (CYP4AII) (CYPIVA11) (Cytochrome P-450HK-omega) (Cytochrome P450HL-omega) (Fatty acid omega-hydroxylase) (Lauric acid omega-hydroxylase) (EC 1.14.15.3)	CYP4A11 CYP4A2	L04751; D26481; S67580; S67581; AF525488; AY369778; AL731892; AL731892; BC041158; X71480;
	Q5TCH4	CP4AM_HUMAN	Cytochrome P450 4A22 (CYPIVA22) (Fatty acid omega-hydroxylase) (Lauric acid omega-hydroxylase) (EC 1.14.15.3)	CYP4A22	AF208532; AY280371; AY280372; AL135960; AL135960; BC148248;
	P13584	CP481_HUMAN	Cytochrome P450 4B1 (EC 1.14.14.1) (CYPIVB1) (Cytochrome P450-HP)	CYP4B1	J02871; X16699; AF491285; AY064485; AY064486; AY151048; DQ518907; AL593856; AL356793; AL593856; AL356793; AL356793; AL593856; AL356793; AL593856; BC017758;
	P78329	CP4F2_HUMAN	Leukotriene-B(4) omega-hydroxylase 1 (EC 1.14.13.30) (CYPIVF2) (Cytochrome P450 4F2) (Cytochrome P450-LTB-omega) (Leukotriene-B(4) 20-monooxygenase 1)	CYP4F2	D26480; U02388; AB015306; AK290790; AF467894; AC005336; BC067437; BC067439; BC067440; AF221943;
	Q08477	CP4F3_HUMAN	Leukotriene-B(4) omega-hydroxylase 2 (EC 1.14.13.30) (CYPIVF3) (Cytochrome P450 4F3)	CYP4F3 LTB4H	D12620; D12621; AB002454; AB002461; AF054821; AY792513;

TABLE 1-continued

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
			(Cytochrome P450-LTB-omega) (Leukotriene-B(4) 20-monooxygenase 2)		
	P98187	CP4F8_HUMAN	Cytochrome P450 4F8 (EC 1.14.14.1) (CYPIVF8)	CYP4F8	AF133298;
	Q9HBI6	CP4F8_HUMAN	Cytochrome P450 4F11 (EC 1.14.14.1) (CYPIVF11)	CYP4F11	AF236085; AC005336; BC016853;
	Q9HCS2	CP4FC_HUMAN	Cytochrome P450 4F12 (EC 1.14.14.1) (CYPIVF12)	CYP4F12 UNQ568/PRO1129	AY008841; AB035130; AB035131; AY358977; AC004523; CH471106;
	Q6NT55	CP4FN_HUMAN	Cytochrome P450 4F22 (EC 1.14.14.—)	CYP4F22	AK096820; BC069351; BC093894; BC093896;
	Q6ZWL3	CP4V2_HUMAN	Cytochrome P450 4V2 (EC 1.14.—.—)	CYP4V2	AY422002; AK122600; AK126473; FJ440682; BC060857;
	Q8N118	CP4X1_HUMAN	Cytochrome P450 4X1 (EC 1.14.14.1) (CYPIVX1)	CYP4X1 UNQ1929/PRO4404	AY358537; AK098065; BC028102;
	Q86W10	CP4Z1_HUMAN	Cytochrome P450 4Z1 (EC 1.14.14.1) (CYPIVZ1)	CYP4Z1 UNQ3060/PRO9882	AY262056; AY358631; AK292175; AL450996; AL135960; AL135960; AL450996;
	Q8N1L4	CP4Z2_HUMAN	Putative cytochrome P450 family member 4Z2	CYP4Z2P	AY696295; AK097373;
	Q16350	CP51A_HUMAN	Lanosterol 14-alpha demethylase (LDM) (EC 1.14.13.70) (CYPLI) (Cytochrome P450 51A1) (Cytochrome P450-14DM) (Cytochrome P45014DM) (Cytochrome P450LI) (Sterol 14-alpha demethylase)	CYP51A1 CYP51	U23942; D55653; U51692; U51684; U51685; U51686; U51687; U51688; U51689; U51690; U51691; AK314205; AK295932; AC000120; CH236949; CH471091; CH471091; BC032322;
	P22680	CP7A1_HUMAN	Cholesterol 7-alpha-monooxygenase (EC 1.14.13.17) (CYPVII (Cholesterol 7-alpha-hydroxylase) (Cytochrome P450 7A1)	CYP7A1 CYP7	X56088; M93133; BC101777; BC112184; L13460; M89647;
	O75881	CP7B1_HUMAN	25-hydroxycholesterol 7-alpha-hydroxylase (Oxysterol 7-alpha-hydroxylase) (EC 1.14.13.100) (Cytochrome P450 7B1)	CYP7B1	AF029403; AF127090; AF176805; AF176800; AF176801; AF176802; AF176803; AF176804; CH471068; BC136574;
	Q9UNU6	CP8B1_HUMAN	7-alpha-hydroxycholest-4-en-3-one 12-alpha-hydroxylase (EC 1.14.13.95) (7-alpha-hydroxy-4-cholesten-3-one 12-alpha-hydroxylase) (CYPVIIIB1) (Cytochrome P450 8B1) (Sterol 12-alpha-hydroxylase)	CYP8B1 CYP12	AF090318; AF090320; AK315330; BC067434; BC067441; BC067442; BC067444;
	Q16647	PTGIS_HUMAN	Prostacyclin synthase (EC 5.3.99.4) (Prostaglandin I2 synthase)	PTGIS CYP8 CYP8A1	D38145; AF297048; AF297049; AF297050; AF297051; AF297052; AL118525; BC101809; BC101811;
	P24557	THAS_HUMAN	Thromboxane-A synthase (TXA synthase) (TXS) (EC 5.3.99.5) (Cytochrome P450 5A1)	TBXAS1 CYP5 CYP5A1	M80647; D34625; L36085; L36075; L36076; L36077; L36078; L36079; L36080; L36081; L36082; L36083; L36084; AF233615; AF233616; AF233617; AF233618; AF233619; AF233620; AF233621; AF233622; AF233623; AF233624; AF233625; BC041157; M74055;
Cyto-chrome b5	Q6P9G0	CB5D1_HUMAN	Cytochrome b5 domain-containing protein 1	CYB5D1	AK057061; AK289520; CH471108; CH471108; CH471108; BC060779;
	O431B9	CYB5B_HUMAN	Cytochrome b5 type B (Cytochrome b5 outer mitochondrial membrane isoform)	CYB5B CYB5M OMB5	AB009282; AK291576; BC004373; BC014431;

TABLE 1-continued

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
	P00167	CYB5_HUMAN	Cytochrome b5 (Microsomal cytochrome b5 type A) (MCB5)	CYB5A CYB5	M22865; M60174; L39945; L39792; L39941; L39942; L39943; L39944; CR456990; CH471117; BC015182;
	Q9UMX5	NENF_HUMAN	Neudesin (Cell immortalization-related protein 2) (Neuron-derived neurotrophic factor) (Secreted protein of unknown function) (SPUF protein)	NENF CIR2 SPUF	AB126219; AF173937; AY762102; AK223135; CH471100; BC008823;
	Q8WUJ1	NEUFC_HUMAN	Neuferricin (Cytochrome b5 domain-containing protein 2)	CYB5D2	AK172844; AK313088; CH471108; CH471108; BC020263; BC051697;
	O00264	PGRC1_HUMAN	Membrane-associated progesterone receptor component 1 (mPR)	PGRMC1 HPR6.6 PGRMC	Y12711; BC034238; AJ249131;
	O15173	PGRC2_HUMAN	Membrane-associated progesterone receptor component 2 (Progesterone membrane-binding protein) (Steroid receptor protein DG6)	PGRMC2 DG6 PMBP	AJ002030; DQ496105; BC016692; BC092478;
	D6RFH4	D6RFH4_HUMAN	Uncharacterized protein	CYB5B	
Cyto-chrome c	P08574	CY1_HUMAN	Cytochrome c1, heme protein, mitochondrial (Complex III subunit 4) (Complex III subunit IV) (Cytochrome b-c1 complex subunit 4) (Ubiquinol-cytochrome-c reductase complex cytochrome c1 subunit) (Cytochrome c-1)	CYC1	M16597; J04444; CR541674; BT019798; DQ300360; BC001006; BC015616; BC020566; X06994;
	P99999	CYC_HUMAN	Cytochrome c	CYCS CYC	M22877; BT006946; AK311836; AL713681; AC007487; CH236948; CH471073; BC005299; BC008475; BC008477; BC009578; BC009579; BC009582; BC009587; BC009602; BC009607; BC014359; BC014361; BC015130; BC016006; BC021994; BC022330; BC067222; BC068464; BC070156; BC070346; BC071761;
Cyto-chrome c oxidases	Q5JTJ3	CA031_HUMAN	Uncharacterized protein C1orf31	C1orf31	AL355472; AL355472; AL355472; BC025793; BC116455;
	P00403	COX2_HUMAN	Cytochrome c oxidase subunit 2 (Cytochrome c oxidase polypeptirie II)	MT-CO2 COII COXII MTCO2	V00662; J01415; X15759; M25171; D38112; U12690; U12691; U12692; U12693; U12694; AF004339; AY339402; AY339403; AY339404; AY339405; AY339406; AY339407; AY339408; AY339409; AY339410; AY339411; AY339412; AY339413; AY339414; AY339415; AY339416; AY339417; AY339418; AY339419; AY339420; AY339421; AY339422; AY339423; AY339424; AY339425; AY339426; AY339427; AY339428; AY339429; AY339430; AY339431; AY339432; AY339433; AY339434; AY339435; AY339436; AY339437; AY339438; AY339439; AY339440; AY339441; AY339442; AY339443; AY339444; AY339445; AY339446; AY339447; AY339448; AY339449; AY339450; AY339451; AY339452; AY339453; AY339454; AY339455; AY339456; AY339457; AY339458; AY339459; AY339460; AY339461; AY339462; AY339463; AY339464; AY339465; AY339466; AY339467; AY339468; AY339469; AY339470; AY339471;

TABLE 1-continued

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
					AY339472; AY339473; AY339474; AY339475; AY339476; AY339477; AY339478; AY339479; AY339480; AY339481; AY339482; AY339483; AY339484; AY339485; AY339486; AY339487; AY339488; AY339489; AY339490; AY339492; AY339493; AY339494; AY339495; AY339496; AY339498; AY339499; AY339500; AY339501; AY339502; AY339503; AY339504; AY339505; AY339506; AY339507; AY339508; AY339509; AY339510; AY339511; AY339512; AY339513; AY339514
	P00414	COX3_HUMAN	Cytochrome c oxidase subunit 3 (Cytochrome c oxidase polypeptide III)	MT-CO3 COIII COXIII MTCO3	J01415; V00662; DQ654394; DQ654395; DQ654396; DQ654397; DQ654398; DQ654399; DQ654400; DQ654401; DQ654402; DQ654403; DQ654404; DQ654405; DQ654406; DQ654407; DQ654408; DQ654409; DQ654410; DQ654411; DQ654412; DQ654413; DQ654414; DQ654415; DQ654416; DQ654417; DQ654418; DQ654419; DQ654420; DQ654421; DQ654422; DQ654423; DQ654424; DQ654425; DQ654426; DQ654427; DQ654428; DQ654429; DQ654430; DQ654431; DQ654432; DQ654433; DQ654434; DQ654435; DQ654436; DQ654437; DQ654438; DQ654439; DQ654440; DQ654441; DQ654442; DQ654443; AF004341;
	P13073	COX41_HUMAN	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial (Cytochrome c oxidase polypeptide IV) (Cytochrome c oxidase subunit IV isoform 1) (COX IV-1)	COX4I1 COX4	M21575; M34600; X54802; U90915; AF005889; AF017115; AF042746; AF042744; AF042745; BT019825; AK311847; CH471114; CH471114; BC008704; BC021236; BC062437;
	Q96KJ9	COX42_HUMAN	Cytochrome c oxidase subunit 4 isoform 2, mitochondrial (Cytochrome c oxidase subunit IV isoform 2) (COX IV-2)	COX4I2 COX4L2	AF257180; AL117381, BC057779;
	P20674	COX5A_HUMAN	Cytochrome c oxidase subunit 5A, mitochondrial (Cytochrome c oxidase polypeptide Va)	COX5A	M22760; DQ987236; DQ987237; CR407649; CH471136; BC024240;
	P10606	COX5B_HUWlAN	Cytochrome c oxidase subunit 5B, mitochondrial (Cytochrome c oxidase polypeptide Vb)	COX5B	M19961; M59250; BC006229; U41284;
	P09669	COX6C_HUMAN	Cytochrome c oxidase subunit 6C (Cytochrome c oxidase polypeptide VIc)	COX6C	X13238; AF067637; AF067636; BT007007; AK311791; CH471060; BC000187;
	P24311	COX7B_HUMAN	Cytochrome c oxidase subunit 7B, mitochondrial (Cytochrome c oxidase polypeptide VIIb)	COX7B	Z14244; BT009767; CR450332; CR542124; AK311879; AL356235; CH471104; BC018386;
	P15954	COX7C_HUMAN	Cytochrome c oxidase subunit 7C, mitochondrial (Cytochrome c oxidase polypeptide VIIc)	COX7C	X16560; AF067639; AF067638; BT007098; BC001005; BC007498;
	O14548	COX7R_HUMAN	Cytochrome c oxidase subunit 7A-related protein, mitochondrial (COX7a-related protein)	COX7A2L COX7AR COX7RP	AB007618; AF127788; AY007643; BT007371; BC005251; BC007095;

TABLE 1-continued

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
			(Cytochrome c oxidase subunit VIIa-related protein) (EB1)		
	O60397	COX7S_HUMAN	Putative Cytochrome c oxidase subunit 7A3, mitochondrial (Cytochrome c oxidase subunit VIIa 3)	COX7A2P2 COX7A3 COX7AL2 COX7AP2	AC004544;
	P10176	COX8A_HUMAN	Cytochrome c oxidase subunit 8A, mitochondrial (Cytochrome c oxidase polypeptide VIII-liver/heart) (Cytochrome c oxidase subunit 8-2)	COX8A COX8 COX8L	J04823; BC063025;
	Q7Z4L0	COX8C_HUMAN	Cytochrome c oxidase subunit 8C, mitochondrial (Cytochrome c oxidase polypeptide 8 isoform 3) (Cytochrome c oxidase polypeptide VIII isoform 3) (COX VIII-3) (Cytochrome c oxidase subunit 8-3)	COX8C	AY161004; BC101125; BC101126;
	P12074	CX6A1_HUMAN	Cytochrome c oxidase subunit 6A1, mitochondrial (Cytochrome c oxidase polypeptidc VIa-liver) (Cytochrome c oxidase subunit VIA-liver) (COX VIa-L)	COX6A1 COX6AL	AK312009; AL021546; BC007723; BC070186; BC107861; X15341;
	Q02221	CX6A2_HUMAN	Cytochrome c oxidase subunit 6A2, mitochondrial (Cytochrome c oxidase polypeptide VIa-heart) (COXVIAH) (Cytochrome c oxidase subunit VIA-muscle) (COX VIa-M)	COX6A2 COX6A COX6AH	M83308; U66875; BC029818;
	P14854	CX6B1_HUMAN	Cytochrome c oxidase subunit 6B1 (Cytochrome c oxidase subunit VIb isoform 1) (COX VIb-1)	COX6B1 COX6B	X13923; X54473; AK312140; BT006945; CR456789; CR542137; AC002115; BC001015; BC002478; X58139;
	Q6YFQ2	CX6B2_HUMAN	Cytochrome c oxidase subunit 6B2 (Cancer/testis antigen 59) (CT59) (Cytochrome c oxidase subunit VIb isoform 2) (COX VIb-2) (Cytochrome c oxidase subunit VIb, testis-specific isoform)	COX6B2	AY152398; AK057427; BC026123; BC100899; BC100900; BC100901; BC100902;
	P24310	CX7A1_HUMAN	Cytochrome c oxidase subunit 7A1, mitochondrial (Cytochrome c oxidase subunit VIIa-heart) (Cytochrome c oxidase subunit VIIa-H) (Cytochrome c oxidase subunit VIIa-muscle) (Cytochrome c oxidase subunit VIIa-M)	COX7A1 COX7AH	M83186; U81524; AC002984; AF037372; AF127789; AD001527; BC002757;
	P14406	CX7A2_HUMAN	Cytochrome c oxidase subunit 7A2, mitochondrial (Cytochrome c oxidase subunit VIIa-liver/heart) (Cytochrome c oxidase subunit VIIa-L) (Cytochrome c oxidase subunit VIIaL)	COX7A2 COX7AL	X15822; AF134406; CR407646; CR542125; AK312154; AL080250; BC101826; BC101828;
	Q8TF08	CX7B2_HUMAN	Cytochrome c oxidase subunit 7B2, mitochondrial (Cytochrome c oxidase polypeptide VIIb2)	COX7B2	AF125109; BC035923; BC107855;

TABLE 1-continued

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
Prosta-glandin G/H synthase	P23219	PGH1_HUMAN	Prostaglandin G/H synthase 1 (EC 1.14.99.1) (Cyclooxygenase-1) (COX-1) (Prostaglandin H2 synthase 1) (PGH synthase 1) (PGHS-1) (PHS 1) (Prostaglandin-endoperoxide synthase 1)	PTGS1 COX1	M31822; M31812; M31813; M31814; M31815; M31816; M31817; M31818; M31819; M31820; M31821; M59979; S78220; S36219; S36271; AF440204; AK290022; AY449688; AL162424; AL359636; AL162424; AL359636; AL162424; AL359636; AL162424; AL359636; AL162424; CH471090; BC029840;
	P35354	PGH2_HUMAN	Prostaglandin G/H synthase 2 (EC 1.14.99.1) (Cyclooxygenase-2) (COX-2) (PHS II) (Prostaglandin H2 synthase 2) (PGH synthase 2) (PGHS-2) (Prostaglandin-endoperoxide synthase 2)	PTGS2 COX2	L15326; M90100; D28235; U04636; AY462100; AY229989; AY382629; AK292167; AL033533; CH471067; BC013734;
Catalases	P04040	CATA_HUMAN	Catalase (EC 1.11.1.6)	CAT	X04085; X04086; X04087; X04088; X04089; X04090; X04091; X04092; X04093; X04094; X04095; X04096; X04076; AY028632; AK291585; AK315350; AY545477; AL035079; CH471064; CH471064; BC110398; BC112217; BC112219; L13609; K02400;
	B4DWK8	B4DWK8_HUMAN	Catalase (EC 1.11.1.6)		AK301577;
Perox-idases	Q9NRD9	DUOX1_HUMAN	Dual oxidase 1 (EC 1.11.1.—) (EC 1.6.3.1) (Large NOX 1) (Long NOX 1) (NADPH thyroid oxidase 1) (Thyroid oxidase 1)	DUOX1 DUOX LNOX1 THOX1	AF230495; AF213465; AK172859; BC114628;
	Q9NRD8	DUOX2_HUMAN	Dual oxidase 2 (EC 1.11.1.—) (EC 1.6.3.1) (Large NOX 2) (Long NOX 2) (NADH/NADPH thyroid oxidase p138-tox) (NADPH oxidase/peroxidase DUOX2) (NADPH thyroid oxidase 2) (Thyroid oxidase 2) (p138 thyroid oxidase)	DUOX2 LNOX2 THOX2	AF230496; AF267981; AF181972;
	P11678	PERE_HUMAN	Eosinophil peroxidase (EPO) (EC 1.11.1.7) [Cleaved into: Eosinophil peroxidase light chain; Eosinophil peroxidase heavy chain]	EPX EPER EPO EPP	M29913; M29904; M29905; M29906; M29907; M29908; M29909; M29910; M29911; M29912; DQ054598; X14346;
	P22079	PERL_HUMAN	Lactoperoxidase (LPO) (EC 1.11.1.7) (Salivary peroxidase) (SPO)	LPO SAPX	U39573; AY324876; BC107166; BC107167; M58151;
	P05164	PERM_HUMAN	Myeloperoxidase (MPO) (EC 1.11.1.7) [Cleaved into: 89 kDa myeloperoxidase; 84 kDa myeloperoxidase; Myeloperoxidase light chain; Myeloperoxidase heavy chain]	MPO	J02694; M17176; M17170; M17171; M17172; M17173; M17174; M17175; X04876; M19507; M19508; M19508; X15377; S56200; DQ088846; CH471109; BC130476; D14466;
	P07202	PERT_HUMAN	Thyroid peroxidase (TPO) (EC 1.11.1.8)	TPO	J02969; J02970; Y00406; M25715; M25702; M25703; M25704; M25705; M25706; M25707; M25708; M25709; M25710; M25711; M25712; M25713; M25714; X17358; M17755; AF439430; AF533528; AY136822; AF533529; AF533530; AF533531; M55702; M55702;
	A1KZ92	PXDNL_HUMAN	Peroxidasin-like protein (EC 1.11.1.7) (Cardiac peroxidase) (Vascular peroxidase 2)	PXDNL VPO2	EU170240; AY877349; AK058200; AK131524; CH471068;

TABLE 1-continued

Human Heme-Binding Proteins

Heme-binding Protein Class	UniProtKB/ Swiss-Prot Accession No.	Entry name	Protein names	Gene names	EMBL Accession No.
	Q92626	PXDN_HUMAN	Peroxidasin homolog (EC 1.11.1.7) (Melanoma-associated antigen MG50) (Vascular peroxidase 1) (p53-responsive gene 2 protein)	PXDN KIAA0230 MG50 PRG2 VPO VPO1	AF200348; EF090903; D86983; CH471053; CH471053; BC098579;
Trans-ferrins	P02787	TRFE_HUMAN	Serotransferrin (Transferrin) (Beta-1 metal-binding globulin) (Siderophilin)	TF PRO1400	M12530; M17611; M17610; M17614; M17612; M17613; S95936; AF288144; AF294270; AF294271; AF288139; AF288140; AF288141; AF288142; AF288143; AY308797; DQ525716; CH471052; BC059367; M21569; M15673; M21570; X04600; AJ252279; M11372; M11361; M11362; M11363; M11364; M11365; M11366; M11367; M11368; M11369; M11370; M11371; AF118063; M12525; U88581; AF058327; M26641;
	P02788	TRFL_HUMAN	Lactotransferrin (Lactoferrin) (EC 3.4.21.—) (Talalactoferrin) [Cleaved into: Kaliocin-1; Lactoferroxin-A; Lactoferroxin-B; Lactoferroxin-C]	LTF LF	X53961; U07643; M93150; M83202; M83205; M73700; AF332168; AY137470; AY165046; AY178998; BC015822; BC015823; BC022347; S52659; X52941; M18642; U95626;
	P08582	TRFM_HUMAN	Melanotransferrin (Melanoma-associated antigen p97) (CD antigen CD228)	MFI2 MAP97	M12154; BC001875; BC002623; BC007550; BC071910;
Nitric Oxide Synthases	P29475	NOS1_HUMAN	Nitric oxide synthase, brain (EC 1.14.13.39) (Constitutive NOS) (NC-NOS) (NOS type I) (Neuronal NOS) (N-NOS) (nNOS) (Peptidyl-cysteine S-nitrosylase NOS1) (bNOS)	NOS1	U17327; U17326; U17299; U17300; U17301; U17302; U17303; U17304; U17305; U17307; U17308; U17309; U17310; U17311; U17312; U17313; U17314; U17315; U17316; U17317; U17318; U17319; U17320; U17321; U17322; U17323; U17324; U17325; D16408; L02881; U31466; AY445095;
	P35228	NOS2_HUMAN	Nitric oxide synthase, indudble (EC 1.14.13.39) (Hepatocyte NOS) (HEP-NOS) (Inducible NO synthase) (Inducible NOS) (iNOS) (NOS type II) (Peptidyl-cysteine S-nitrosylase NOS2)	NOS2 NOS2A	L24553; L09210; X73029; U05810; U31511; D26525; U20141; AF068236; AB022318; DQ060518; EU332854; BC130283; BC144126; S75615;
	P29474	NOS3_HUMAN	Nitric oxide synthase, endothelial (EC 1.14.13.39) (Constitutive NOS) (cNOS) (EC-NOS) (Endothelial NOS) (eNOS) (NOS type III) (NOSIII)	NOS3	M93718; M95296; L10709; L10693; L10694; L10695; L10696; L10697; L10698; L10699; L10700; L10701; L10702; L10703; L10704; L10705; L10706; L10707; L10708; L26914; X76303; X76304; X76305; X76306; X76307; X76308; X76309; X76310; X76311; X76312; X76313; X76314; X76315; X76316; D26607; AF400594; AK292928; AK315213; AK223636; AF519768; EU332855; CH471173; BC063294; BC069465; L23210; S80791;
	O75713	O75713_HUMAN	Neuronal nitric-oxide synthase isoform mu (EC 1.14.13.39) (Fragment)	nNOSmu	AJ004918
Heme-oxy-genases	P09601	HMOX1_HUMAN	Heme oxygenase 1 (HO-1) (EC 1.14.99.3)	HMOX1 HO HO1	X06985; CR456505; AY460337; Z82244; M23041; X14782;
	P30519	HMOX2_HUMAN	Heme oxygenase 2 (HO-2) (EC 1.14.99.3)	HMOX2 HO2	D21243; S34389; BT019788; AY771350; CH471112; CH471112; BC002396; AF051306;

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TABLE 2

Lignin Peroxidases

UniProtKB/ Swiss-Prot Accession	Entry name	Protein names	Gene names	EMBL Accession No.	Protein family	Organism
P28313	PER_ARTRA	Peroxidase (EC 1.11.1.7)		D63792;	Peroxidase family, Ligninase subfamily	*Arthromyces ramosus*
P28314	PER_COPCI	Peroxidase (EC 1.11.1.7)	CIP1	X69457; X70789;	Peroxidase family, Ligninase subfamily	*Coprinopsis cinerea* (Inky cap fungus) (*Hormographiella aspergillata*)
A8NK72	PER_COPC7	Peroxidase (EC 1.11.1.7)	CIP1 CC1G_02104	AACS02000010;	Peroxidase family, Ligninase subfamily	*Coprinopsis cinerea* (strain Okayama-7/130/ FGSC 9003) (Inky cap fungus) (*Hormographiella aspergillata*)
P49012	LIG2_PHACH	Ligninase LG2 (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase)	GLG2 LIP2	M74229; M92644;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
P21764	LIG3_PHACH	Ligninase LG3 (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase)	GLG3 LIP	X51590;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
P11542	LIG4_PHACH	Ligninase H2 (EC 1.11.1.14) (Diarylpropane peroxidase) (LG4) (Lignin peroxidase)	GLG4 LIP2	X15599; M18743;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
P11543	LIG5_PHACH	Ligninase LG5 (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase)	GLG5 LIP6	M18794; X55343; M63496;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
P50622	LIG6_PHACH	Ligninase LG6 (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase)	GLG6	M80213;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
P06181	LIG8_PHACH	Ligninase H8 (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase)	LPOA	M37701; Y00262; M27401; M27884;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
P31837	LIGA_PHACH	Ligninase A (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase)	LIPA LPOB	X54257; M37701;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
P31838	LIGB_PHACH	Ligninase B (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase)	LIPB	X54257;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
Q02567	PEM1_PHACH	Manganese peroxidase 1 (MnP-1) (MnP1) (EC 1.11.1.13) (Manganese peroxidase isozyme 1) (Peroxidase manganese-dependent 1) (Peroxidase manganese-dependent I)	MNP1	M60672; M77513; J04624;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
P78733	PEM3_PHACH	Manganese peroxidase H3 (EC 1.11.1.13) (Peroxidase manganese-dependent H3)		U10306;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)

TABLE 2-continued

Lignin Peroxidases						
UniProtKB/ Swiss-Prot Accession	Entry name	Protein names	Gene names	EMBL Accession No.	Protein family	Organism
P19136	PEM4_PHACH	Manganese peroxidase H4 (EC 1.11.1.13) (MP-I) (Peroxidase manganese-dependent H4)		J04980;	Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
Q9URB1	PEM5_PHACH	Manganese peroxidase H5 (EC 1.11.1.13) (Peroxidase manganese-dependent H5) (Fragment)			Peroxidase family, Ligninase subfamily	*Phanerochaete chrysosporium* (White-rot fungus) (*Sporotrichum pruinosum*)
P20010	LIG_PHLRA	Ligninase-3 (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase) (Ligninase III)			Peroxidase family, Ligninase subfamily	*Phlebia radiata* (White-rot fungus)
Q70LM3	PEM2_PHLRA	Manganese peroxidase 2 (MnP2) (EC 1.11.1.13) (Manganese peroxidase isozyme 2)	mnp2	AJ315701; AJ566199;	Peroxidase family, Ligninase subfamily	*Phlebia radiata* (White-rot fungus)
Q96TS6	PEM3_PHLRA	Manganese peroxidase 3 (MnP3) (EC 1.11.1.13) (Manganese peroxidase isozyme 3)	mnp3	AJ310930; AJ566200;	Peroxidase family, Ligninase subfamily	*Phlebia radiata* (White-rot fungus)
Q9UR19	VPL1_PLEER	Versatile peroxidase VPL1 (EC 1.11.1.16) (Versatile liquid phase peroxidase 1)	vpl1	AF007221; AF007223;	Peroxidase family, Ligninase subfamily	*Pleurotus eryngii* (Boletus of the steppes)
O94753	VPL2_PLEER	Versatile peroxidase VPL2 (EC 1.11.1.16) (Versatile liquid phase peroxidase 2)	vpl2	AF007222; AF007224;	Peroxidase family, Ligninase subfamily	*Pleurotus eryngii* (Boletus of the steppes)
Q9UVP6	VPS1_PLEER	Versatile peroxidase VPS1 (EC 1.11.1.16) (Versatile solid phase peroxidase 1)	vps1 ps1	AF175710;	Peroxidase family, Ligninase subfamily	*Pleurotus eryngii* (Boletus of the steppes)
C0IW58	LNP_TAICA	Low-redox potential peroxidase (EC 1.11.1.7) (Putative ligninolytic peroxidase)	LnP	EU289404; EU526903;	Peroxidase family, Ligninase subfamily	*Taiwanofungus camphoratus* (Poroid brown-rot fungus) (*Antrodia camphorata*)
P20011	LIGA_TRAVE	Ligninase A (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase) (Fragment)			Peroxidase family, Ligninase subfamily	*Trametes versicolor* (White-rot fungus) (*Coriolus versicolor*)
P20012	LIGB_TRAVE	Ligninase B (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase) (Fragment)			Peroxidase family, Ligninase subfamily	*Trametes versicolor* (White-rot fungus) (*Coriolus versicolor*)
P20013	LIGC_TRAVE	Ligninase C (EC 1.11.1.14) (Diarylpropane peroxidase) (Lignin peroxidase)		M64993;	Peroxidase family, Ligninase subfamily	*Trametes versicolor* (White-rot fungus) (*Coriolus versicolor*)

(excitation at 397 nm) and resonance Raman spectroscopy. As demonstrated herein, recombinant heme-binding proteins generated using the methods of the present invention show no evidence of free base porphryin incorporation.

[0025] The recombinant ferrochelatase protein or polypeptide used in the methods of the present invention includes any recombinant ferrochelatase, or polypeptide thereof that is capable of catalyzing the insertion of ferrous iron into protoporphyrin IX to form protoheme (i.e., enzyme classification (EC) no. 4.99.1.1). Well over 400 ferrochelatases from both prokaryotes and eukaryotes have been characterized and are known in the art (see e.g., UniProtKB, (GenBank). Table 3

below provides a listing of 429 known ferrochelatase enzymes from the UniProtKB database identified by UniProtKB/Swiss-Pro Accession number, protein name, gene name, organism, EC number, and EMBL accession number. Each of the ferrochelatases listed in Table 3 is suitable for use in the present invention. The UniProtKB/Swiss Prot and EMBL accession numbers, along with the corresponding amino acid and nucleotide sequence information for each entry in Table 3 is hereby incorporated by reference. Selection of an appropriate ferrochelatase to use when carrying out the methods of the present invention is based on the recombinant heme-binding protein being produced and/or the chosen expression system.

[0026] It is to be understood that the present invention contemplates the use of any bacterial, archaeal, fungal, plant, and animal ferrochelatase known in the art. The present invention also contemplates the use of polypeptide fragments of ferrochelatase which retain iron insertion activity (i.e., suitable proteins or polypeptides have an enzyme classification of 4.99.1.1). Methods of analyzing and measuring the enzyme activity of ferrochelatase are well known in the art (see e.g., Miyamoto et al., "Overproduction, Purification, and Characterization of Ferrochelatase from *Escherichia coli," J. Biochem.* 115:545-551 (1994) and Taketani et al., "Rat Liver Ferrochelatase," *J. Biol. Chem.,* 256(24):12748-53 (1981), which are hereby incorporated by reference in their entirety). Therefore, suitable ferrochelatase proteins or polypeptides not identified in Table 3 can be readily identified by one of skill in the art using these enzyme activity assays.

TABLE 3

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
P42043	HEMH1_ARATH	HEM15 At5g26030 T1N24.17	*Arabidopsis thaliana* (Mouse-ear cress)	4.99.1.1	Ferrochelatase-1, chloroplastic/mitochondrial (EC 4.99.1.1) (Ferrochelatase I) (Heme synthase 1) (Protoheme ferro-lyase 1)	X73417; Y13382; AF149413;
Q81U22	HEMH1_BACAN	hemH1 hemH-1 BA_1071 GBAA_1071 BAS1000	*Bacillus anthracis*	4.99.1.1	Ferrochelatase 1 (EC 4.99.1.1) (Heme synthase 1) (Protoheme ferro-lyase 1)	AE016879; AE017334; AE017225;
Q73C98	HEMH1_BACC1	hemH1 BCE_1168	*Bacillus cereus* (strain ATCC 10987)	4.99.1.1	Ferrochelatase 1 (EC 4.99.1.1) (Heme synthase 1) (Protoheme ferro-lyase 1)	AE017194;
Q81GW5	HEMH1_BACCR	hemH1 BC_1069	*Bacillus cereus* (strain ATCC 14579/ DSM 31)	4.99.1.1	Ferrochelatase 1 (EC 4.99.1.1) (Heme synthase 1) (Protoheme ferro-lyase 1)	AE016877;
Q63ES4	HEMH1_BACCZ	hemH1 BCE33L0987	*Bacillus cereus* (strain ZK/E33L)	4.99.1.1	Ferrochelatase 1 (EC 4.99.1.1) (Heme synthase 1) (Protoheme ferro-lyase 1)	CP000001;
Q6HM97	HEMH1_BACHK	hemH1 BT9727_0985	*Bacillus thuringiensis* subsp. *konkukian*	4.99.1.1	Ferrochelatase 1 (EC 4.99.1.1) (Heme synthase 1) (Protoheme ferro-lyase 1)	AE017355;
Q69TB1	HEMH1_ORYSJ	Os09g0297000 LOC_Os09g12560 P0592C05.24	*Oryza sativa* subsp. *japonica* (Rice)	4.99.1.1	Ferrochelatase-1, chloroplastic (EC 4.99.1.1) (Ferrochelatase I) (Heme synthase 1) (Protoheme ferro-lyase 1)	AP004756; AP008215; AK068174;
Q8EFF4	HEMH1_SHEON	hemH1 hemH-1 SO_2019	*Shewanella oneidensis*	4.99.1.1	Ferrochelatase 1 (EC 4.99.1.1) (Heme synthase 1) (Protoheme ferro-lyase 1)	AE014299;
O04921	HEMH2_ARATH	At2g30390 T06B20.24 T9D9.1	*Arabidopsis thaliana* (Mouse-ear cress)	4.99.1.1	Ferrochelatase-2, chloroplastic (EC 4.99.1.1) (Ferrochelatase II) (Heme synthase 2) (Protoheme ferro-lyase 2)	Y13156; U93215; AC002338; BT000465; BT008877;
Q81TU9	HEMH2_BACAN	hemH2 hemH-2 BA_1158 GBAA_1158 BAS1075	*Bacillus anthracis*	4.99.1.1	Ferrochelatase 2 (EC 4.99.1.1) (Heme synthase 2) (Protoheme ferro-lyase 2)	AE016879; AE017334; AE017225;
Q73C08	HEMH2_BACC1	hemH2 BCE_1260	*Bacillus cereus* (strain ATCC 10987)	4.99.1.1	Ferrochelatase 2 (EC 4.99.1.1) (Heme synthase 2) (Protoheme ferro-lyase 2)	AE017194;
Q81GN7	HEMH2_BACCR	hemH2 BC_1154	*Bacillus cereus* (strain ATCC 14579/ DSM 31)	4.99.1.1	Ferrochelatase 2 (EC 4.99.1.1) (Heme synthase 2) (Protoheme ferro-lyase 2)	AE016877;
Q63EK7	HEMH2_BACCZ	hemH2 BCE33L1054	*Bacillus cereus* (strain ZK/E33L)	4.99.1.1	Ferrochelatase 2 (EC 4.99.1.1) (Heme synthase 2) (Protoheme ferro-lyase 2)	CP000001;
Q6HM28	HEMH2_BACHK	hemH2 BT9727_1056	*Bacillus thuringiensis* subsp. *konkukian*	4.99.1.1	Ferrochelatase 2 (EC 4.99.1.1) (Heme synthase 2) (Protoheme ferro-lyase 2)	AE017355;
Q0DIV0	HEMH2_ORYSJ	Os05g0361200 LOC_Os05g29760 P0530H10.9 P0692D12.2	*Oryza sativa* subsp. *japonica* (Rice)	4.99.1.1	Ferrochelatase-2, chloroplastic (EC 4.99.1.1) (Ferrochelatase II) (Heme synthase 2) (Protoheme ferro-lyase 2)	AC134348; AC135929; AP008211; AK073873;
Q8EBZ7	HEMH2_SHEON	hemH2 hemH-2 SO_3348	*Shewanella oneidensis*	4.99.1.1	Ferrochelatase 2 (EC 4.99.1.1) (Heme synthase 2) (Protoheme ferro-lyase 2)	AE014299;
Q6F7N0	HEMH_ACIAD	hemH ACIAD3255	*Acinetobacter* sp. (strain ADP1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CR543861;
B7H0X1	HEMH_ACIB3	hemH ABBFA_003155	*Acinetobacter baumannii* (strain AB307-0294)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001172;
B7I4A8	HEMH_ACIB5	hemH AB57_0460	*Acinetobacter baumannii* (strain AB0057)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001182;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
B2I2R9	HEMH_ACIBC	hemH ACICU_00392	*Acinetobacter baumannii* (strain ACICU)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000863;
B0VLE0	HEMH_ACIBS	hemH ABSDF3128	*Acinetobacter baumannii* (strain SDF)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU468230;
A3M1P7	HEMH_ACIBT	hemH A1S_0382	*Acinetobacter baumannii* (strain ATCC 17978/NCDC KC 755)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000521;
B0VEC9	HEMH_ACIBY	hemH ABAYE3393	*Acinetobacter baumannii* (strain AYE)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU459141;
C1F1C7	HEMH_ACIC5	hemH ACP_2433	*Acidobacterium capsulatum* (strain ATCC 51196/DSM 11244/JCM 7670)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001472;
A3N3M9	HEMH_ACTP2	hemH APL_1937	*Actinobacillus pleuropneumoniae* serotype 5b (strain L20)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000569;
B3H307	HEMH_ACTP7	hemH APP7_2025	*Actinobacillus pleuropneumoniae* serotype 7 (strain AP76)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001091;
B0BTL7	HEMH_ACTPJ	hemH APJL_1983	*Actinobacillus pleuropneumoniae* serotype 3 (strain JL03)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000687;
A0KL51	HEMH_AERHH	hemH AHA_2488	*Aeromonas hydrophila* subsp. *hydrophila* (strain ATCC 7966/NCIB 9240)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000462;
Q8U9F7	HEMH_AGRT5	hemH Atu3771 AGR_L_2129	*Agrobacterium tumefaciens* (strain C58/ATCC 33970)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE007870;
B9JS40	HEMH_AGRVS	hemH Avi_3712	*Agrobacterium vitis* (strain S4/ATCC BAA-846) (*Rhizobium vitis* (strain S4))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000633;
Q0VSV6	HEMH_ALCBS	hemH ABO_0294	*Alcanivorax borkumensis* (strain SK2/ ATCC 700651/DSM 11573)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM286690;
B6EHK1	HEMH_ALISL	hemH VSAL_I0816	*Alilvibrio salmonicida* (strain LFI1238) (*Vibrio salmonicida* (strain LFI1238))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	FM178379;
B4S1G4	HEMH_ALTMD	hemH MADE_00654	*Alteromonas macieodii* (strain DSM 17117/Deep ecotype)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001103;
Q3MCT9	HEMH_ANAVT	hemH Ava_1574	*Anabaena variabilis* (strain ATCC 29413/PCC 7937)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000117;
B7GF12	HEMH_ANOFW	hemH Aflv_2279	*Anoxybacillus flavithermus* (strain DSM 21510/WK1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000922;
O67083	HEMH_AQUAE	hemH aq_948	*Aquifex aeolicus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE000657;
Q5P1H1	HEMH_AROAE	hemH AZOSEA27180 ebA4802	*Aromatoleum aromaticum* (strain EbN1) (*Azoarcus* sp. (strain EbN1))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CR555306;
A1K8P4	HEMH_AZOSB	hemH azo2582	*Azoarcus* sp. (strain BH72)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM406670;
C1DEU1	HEMH_AZOVD	hemH Avin_41370	*Azotobacter vinelandii* (strain DJ/ ATCC BAA-1303)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001157;
Q9KDK9	HEMH_BACHD	hemH BH1203	*Bacillus halodurans*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000004;
A8FBM6	HEMH_BACP2	hemH BPUM_0959	*Bacillus pumilus* (strain SAFR-032)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000813;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
Q5WHT1	HEMH_BACSK	hemH ABC1539	*Bacillus clausii* (strain KSM-K16)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP006627;
P32396	HEMH_BACSU	hemH hemF BSU10130	*Bacillus subtilis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	M97208; Y14083; AL009126;
A9VI99	HEMH_BACWK	hemH BcerKBAB4_0990	*Bacillus weihenstephanensis* (strain KBAB4)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000903;
Q6MHT3	HEMH_BDEBA	hemH Bd3456	*Bdellovibrio bacteriovorus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX842655;
Q7WGI0	HEMH_BORBR	hemH BB3938	*Bordetella bronchiseptica* (*Alcaligenes bronchisepticus*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX640449;
Q7W515	HEMH_BORPA	hemH BPP3489	*Bordetella parapertussis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX640433;
Q7VVX8	HEMH_BORPE	hemH BP2503	*Bordetella pertussis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX640418;
P22600	HEMH_BOVIN	FECH	*Bos taurus* (Bovine)	4.99.1.1	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	L34173;
P28602	HEMH_BRAJA	hemH bll7752	*Bradyrhizobium japonicum*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	M92427; BA000040;
A5EBP8	HEMH_BRASB	hemH BBta_1357	*Bradyrhizobium* sp. (strain BTAi1/ ATCC BAA-1182)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000494;
B2SCP3	HEMH_BRUA1	hemH BAbS19_II00700	*Brucella abortus* (strain S19)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000888;
Q2YIS9	HEMH_BRUA2	hemH BAB2_0075	*Brucella abortus* (strain 2308)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM040265;
P0A3D8	HEMH_BRUAB	hemH BruAb2_0076	*Brucella abortus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AF358663; AY027659; AE017224;
A9MDJ3	HEMH_BRUC2	hemH BCAN_B0079	*Brucella canis* (strain ATCC 23365/ NCTC 10854)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000873;
C0RK28	HEMH_BRUMB	hemH BMEA_B0080	*Brucella melitensis* biotype 2 (strain ATCC 23457)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001489;
P0A3D6	HEMH_BRUME	hemH BMEII0018	*Brucella melitensis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE008918;
A5VTJ7	HEMH_BRUO2	hemH BOV_A0071	*Brucella ovis* (strain ATCC 25840/ 63/290/NCTC 10512)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000709;
A9WXE2	HEMH_BRUSI	hemH BSUIS_B0081	*Brucella suis* (strain ATCC 23445/ NCTC 10510)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000912;
P0A3D7	HEMH_BRUSU	hemH BRA0076	*Brucella suis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE014292;
B1YTJ5	HEMH_BURA4	hemH BamMC406_0664	*Burkholderia ambifaria* (strain MC40-6)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001025;
Q1BYX9	HEMH_BURCA	hemH Bcen_0262	*Burkholderia cenocepacia* (strain AU 1054)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000378;
B1JW14	HEMH_BURCC	hemH Bcenmc03_0716	*Burkholderia cenocepacia* (strain MC0-3)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000958;
A0K4S2	HEMH_BURCH	hemH Bcen2424_0746	*Burkholderia cenocepacia* (strain HI2424)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000458;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
Q0BI24	HEMH_BURCM	hemH Bamb_0640	*Burkholderia ambifaria* (strain ATCC BAA-244/AMMD) (*Burkholderia cepacia* (strain AMMD))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000440;
A3MNA4	HEMH_BURM7	hemH BMA10247_2211	*Burkholderia mallei* (strain NCTC 10247)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000548;
A2S570	HEMH_BURM9	hemH BMA10229_A1104	*Burkholderia mallei* (strain NCTC 10229)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000546;
Q62HD1	HEMH_BURMA	hemH BMA2330	*Burkholderia mallei* (*Pseudomonas mallei*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000010;
A1V0U1	HEMH_BURMS	hemH BMASAVP1_A0495	*Burkholderia mallei* (strain SAVP1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000526;
A3NYY2	HEMH_BURP0	hemH BURPS1106A_3316	*Burkholderia pseudomallei* (strain 1106a)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000572;
Q3JP06	HEMH_BURP1	hemH BURPS1710b_3326	*Burkholderia pseudomallei* (strain 1710b)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000124;
A3ND73	HEMH_BURP6	hemH BURPS668_3283	*Burkholderia pseudomallei* (strain 668)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000570;
B2JGE7	HEMH_BURP8	hemH Bphy_2502	*Burkholderia phymatum* (strain DSM 17167/STM815)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001043;
B2SXB8	HEMH_BURPP	hemH Bphyt_0733	*Burkholderia phytofirmans* (strain DSM 17436/PsJN)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001052;
Q63R43	HEMH_BURPS	hemH BPSL2831	*Burkholderia pseudomallei* (*Pseudomonas pseudomallei*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX571965;
Q39JD2	HEMH_BURS3	hemH Bcep18194_A3835	*Burkholderia* sp. (strain 383) (*Burkholderia cepacia* (strain ATCC 17760/NCIB 9086/R18194))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000151;
Q2SYZ9	HEMH_BURTA	hemH BTH_I1303	*Burkholderia thailandensis* (strain E264/ATCC 700388/DSM 13276/ CIP 106301)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000086;
A4JBR6	HEMH_BURVG	hemH Bcep1808_0707	*Burkholderia vietnamiensis* (strain G4/ LMG 22486) (*Burkholderia cepacia* (strain R1808))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000614;
Q145F5	HEMH_BURXL	hemH Bxeno_A0496 Bxe_A3965	*Burkholderia xenovorans* (strain LB400)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000270;
A7ZE60	HEMH_CAMC1	hemH Ccon26_12120 CCC13826_0053	*Campylobacter concisus* (strain 13826)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000792;
A0RNU4	HEMH_CAMFF	hemH CFF8240_0703	*Campylobacter fetus* subsp. *fetus* (strain 82-40)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000487;
A8FKS9	HEMH_CAMJ8	hemH C8J_0467	*Campylobacter jejuni* subsp. *jejuni* serotype O:6 (strain 81116/NCTC 11828)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000814;
A7H4N6	HEMH_CAMJD	hemH JJD26997_1433	*Campylobacter jejuni* subsp. *doylei* (strain ATCC BAA-1458/RM4099/ 269.97)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000768;
Q9PI08	HEMH_CAMJE	hemH Cj0503c	*Campylobacter jejuni*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL111168;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
A1VYL5	HEMH_CAMJJ	hemH CJJ81176_0531	*Campylobacter jejuni* subsp. *jejuni* serotype O:23/36 (strain 81-176)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000538;
Q5HVR0	HEMH_CAMJR	hemH CJE0610	*Campylobacter jejuni* (strain RM1221)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000025;
B8GW40	HEMH_CAUCN	hemH CCNA_03878	*Caulobacter crescentus* (strain NA1000/CB15N)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001340;
P57777	HEMH_CAUCR	hemH CC_3762	*Caulobacter crescentus* (*Caulobacter vibrioides*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AF184071; AE005673;
O42479	HEMH_CHICK	FECH	*Gallus gallus* (Chicken)	4.99.1.1	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	U68033;
Q5L6X6	HEMH_CHLAB	hemH CAB136	*Chlamydophila abortus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CR848038;
Q824K8	HEMH_CHLCV	hemH CCA_00137	*Chlamydophila caviae*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE015925;
Q252Z7	HEMH_CHLFF	hemH CF0869	*Chlamydophila felis* (strain Fe/C-56)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP006861;
Q9PJQ6	HEMH_CHLMU	hemH TC_0772	*Chlamydia muridarum*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE002160;
Q9Z7V1	HEMH_CHLPN	hemH CPn_0603 CP_0144 CpB0627	*Chlamydia pneumoniae* (*Chlamydophila pneumoniae*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE001363; AE002161; BA000008; AE009440;
B0B858	HEMH_CHLT2	hemH CTL0746	*Chlamydia trachomatis* serovar L2 (strain 434/Bu/ATCC VR-902B)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM884176;
Q3KLL2	HEMH_CHLTA	hemH CTA_0532	*Chlamydia trachomatis* serovar A (strain HAR-13/ATCC VR-571B)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000051;
B0BCC3	HEMH_CHLTB	hemH CTLon_0741	*Chlamydia trachomatis* serovar L2b (strain UCH-1/proctitis)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM884177;
O84492	HEMH_CHLTR	hemH CT_485	*Chlamydia trachomatis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE001273;
Q7NV65	HEMH_CHRVO	hemH CV_2480	*Chromobacterium violaceum*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE016825;
Q6NH66	HEMH_CORDI	hemH DIP1280	*Corynebacterium diphtheriae*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX248357;
Q8FTB1	HEMH_COREF	hemH CE1658	*Corynebacterium efficiens*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000035;
A4QEC1	HEMH_CORGB	hemH cgR_1595	*Corynebacterium glutamicum* (strain R)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP009044;
Q8NQA1	HEMH_CORGL	hemH Cgl1537 cg1734	*Corynebacterium glutamicum* (*Brevibacterium flavum*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000036; BX927152;
B6J5R6	HEMH_COXB1	hemH CbuK_1987	*Coxiella bumetii* (strain CbuK_Q154) (*Coxiella bumetii* (strain Q154))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001020;
B6J386	HEMH_COXB2	hemH CbuG_1969	*Coxiella bumetii* (strain CbuG_Q212) (*Coxiella bumetii* (strain Q212))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001019;
A9KGX9	HEMH_COXBN	hemH CBUD_2060	*Coxiella bumetii* (strain Dugway 5J108-111)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000733;
Q83FA4	HEMH_COXBU	hemH CBU_0042	*Coxiella burnetii*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE016828;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
P42044	HEMH_CUCSA	HEMH	*Cucumis sativus* (Cucumber)	4.99.1.1	Ferrochelatase-2, chloroplastic (EC 4.99.1.1) (Ferrochelatase II) (Heme synthase 2) (Protoheme ferro-lyase 2)	D26106;
Q0KCJ6	HEMH_CUPNH	hemH H16_A1134	*Cupriavidus necator* (strain ATCC 17699/H16/DSM 428/Stanier 337) (*Ralstonia eutropha*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM260479;
Q473L7	HEMH_CUPPJ	hemH Reut_A1037	*Cupriavidus pinatubonensis* (strain JMP134/LMG 1197) (*Alcaligenes eutrophus*) (*Ralstonia eutropha*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000090;
B3R447	HEMH_CUPTR	hemH RALTA_A1114	*Cupriavidus taiwanensis* (strain R1/ LMG 19424) (*Ralstonia taiwanensis* (strain LMG 19424))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU633749;
B8HK77	HEMH_CYAP4	hemH Cyan7425_4517	*Cyanothece* sp. (strain PCC 7425/ ATCC 29141)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001344;
B7KGB9	HEMH_CYAP7	hemH PCC7424_2167	*Cyanothece* sp. (strain PCC 7424) (*Synechococcus* sp. (strain ATCC 29155))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001291;
B7K399	HEMH_CYAP8	hemH PCC8801_0320	*Cyanothece* sp. (strain PCC 8801) (*Synechococcus* sp. (strain PCC 8801/ RF-1))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001287;
Q47HJ6	HEMH_DECAR	hemH Daro_0929	*Dechloromonas aromatica* (strain RCB)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP900089;
Q9RV98	HEMH_DEIRA	hemH DR_1131	*Deinococcus radiodurans*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE000513;
Q6APB0	HEMH_DESPS	hemH DP1085	*Desulfotalea psychrophila*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CR522870;
Q54IA8	HEMH_DICDI	hemH DD8_G0288891	*Dictyostelium discoideum* (Slime mold)	4.99.1.1	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AAFI02000126;
Q9V9S8	HEMH_DROME	Fech CG2098	*Drosophila melanogaster* (Fruit fly)	4.99.1.1	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AF076220; AE014297; AE014297; AE014297; AY058251; BT001392; BT001878;
A7ZIN5	HEMH_ECO24	hemH EcE24377A_0515	*Escherichia coli* O139:H28 (strain E24377A/ETEC)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000800;
B7UKF5	HEMH_ECO27	hemH E2348_C_0410	*Escherichia coli* O127:H6 (strain E2348/69/EPEC)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	FM180568;
B7MDZ7	HEMH_ECO45	hemH ECS88_0472	*Escherichia coil* O45:K1 (strain S88/ ExPEC)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU928161;
B7L7A0	HEMH_ECO55	hemH EC55989_0488	*Escherichia coli* (strain 55989/EAEC)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU928145;
Q8XD39	HEMH_ECO57	hemH Z0592 ECs0528	*Escherichia coli* O157:H7	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE005174; BA000007;
B5Z3Y6	HEMH_ECO5E	hemH ECH74115_0568	*Escherichia coli* O157:H7 (strain EC4115/EHEC)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001164;
B7NIF5	HEMH_ECO71	hemH ECIAI39_0196	*Escherichia coli* O7:K1 (strain IAI39/ ExPEC)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU928164;

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Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
B7MQJ0	HEMH_ECO81	hemH ECED1_0498	*Escherichia coli* O81 (strain ED1a)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU928162;
B7M3W7	HEMH_ECO8A	hemH ECIAI1_0478	*Escherichia coli* O8 (strain IAI1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU928160;
C4ZUS9	HEMH_ECOBW	hemH BWG_0356	*Escherichia coli* (strain K12/MC4100/ BW2952)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001396;
B1XFR2	HEMH_ECODH	hemH ECDH10B_0431	*Escherichia coli* (strain K12/DH10B)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000948;
A7ZXD3	HEMH_ECOHS	hemH EcHS_A0552	*Escherichia coli* O9:H4 (strain HS)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000802;
A1A8E1	HEMH_ECOK1	hemH Ecok1_04370 APECO1_1540	*Escherichia coli* O1:K1/APEC	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000468;
Q0TKG6	HEMH_ECOL5	hemH ECP_0536	*Escherichia coli* O6:K15:H31 (strain 536/UPEC)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000247;
Q8FK83	HEMH_ECOL6	hemH c0595	*Escherichia coli* O6	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE014075;
B1IZB9	HEMH_ECOLC	hemH EcolC_3141	*Escherichia coli* (strain ATCC 8739/ DSM 1576/Crooks)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000946;
P23871	HEMH_ECOLI	hemH popA visA b0475 JW0464	*Escherichia coli* (strain K12)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	D90259; U82664; U00096; AP009048;
B7N928	HEMH_ECOLU	hemH ECUMN_0514	*Escherichia coli* O17:K52:H18 (strain UMN026/ExPEC)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU928163;
B6I0C7	HEMH_ECOSE	hemH ECSE_0500	*Escherichia coli* (strain SE11)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP009240;
B1LJN3	HEMH_ECOSM	hemH EcSMS35_0520	*Escherichia coli* (strain SMS-3-5/ SECEC)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000970;
Q1RF60	HEMH_ECOUT	hemH UTI89_C0503	*Escherichia coli* (strain UTI89/UPEC)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000243;
C5BD17	HEMH_EDWI9	hemH NT01EI_1124	*Edwardsiella ictaluri* (strain 93-146)	499.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001600;
A4W7F9	HEMH_ENT38	hemH Ent638_0955	*Enterobacter* sp. (strain 638)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000653;
Q833G5	HEMH_ENTFA	hemH EF_1989	*Enterococcus faecalis* (*Streptococcus faecalis*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE016830;
A7MNM8	HEMH_ENTS8	hemH ESA_02788	*Enterobacter sakazakii* (strain ATCC BAA-894)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000783;
Q6D7Z4	HEMH_ERWCT	hemH ECA1181	*Erwinia carotovora* subsp. *atroseptica* (*Pectobacterium atrosepticum*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX950851;
B2VHX1	HEMH_ERWT9	hemH ETA_24640	*Erwinia tasmaniensis* (strain DSM 17950/Et1/99)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU468135;
B7LV14	HEMH_ESCF3	hemH EFER_0525	*Escherichia fergusonii* (strain ATCC 35469/DSM 13698/CDC 0568-73)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU928158;
Q0RH75	HEMH_FRAAA	hemH FRAAL4516	*Frankia alni* (strain ACN14a)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CT573213;
Q14H85	HEMH_FRAT1	hemH FTF1138	*Francisella tularensis* subsp. *tularensis* (strain FSC 198)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM286280;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
A7NBJ3	HEMH_FRATF	hemH FTA_0870	*Francisella tularensis* subsp. *holarctica* (strain FTNF002-00/FTA)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000803;
Q2A406	HEMH_FRATH	hemH FTL_0821	*Francisella tularensis* subsp. *holarctica* (strain LVS)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM233362;
B2SDE2	HEMH_FRATM	hemH FTM_1306	*Francisella tularensis* subsp. *mediasiatica* (strain FSC147)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000915;
A0Q6Z1	HEMH_FRATN	hemH FTN_1120	*Francisella tularensis* subsp. *novicida* (strain U112)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000439;
Q0BME1	HEMH_FRATO	hemH FTH_0813	*Francisella tularensis* subsp. *holarctica* (strain OSU18)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000437;
Q5NFT3	HEMH_FRATT	hemH FTT_1138	*Francisella tularensis* subsp. *tularensis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AJ749949;
A4IYH5	HEMH_FRATW	hemH FTW_1172	*Francisella tularensis* subsp. *tularensis* (strain WY96-3418)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000608;
B5EJ44	HEMH_GEOBB	hemH Gbem_0039	*Geobacter bemidjiensis* (strain Bem/ ATCC BAA-1014/DSM 16622)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001124;
Q5L283	HEMH_GEOKA	hemH GK0662	*Geobacillus kaustophilus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000043;
Q39ZQ5	HEMH_GEOMG	hemH Gmet_0019	*Geobacter metallireducens* (strain GS-15/ATCC 53774/DSM 7210)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000148;
B9M326	HEMH_GEOSF	hemH Geob_1076	*Geobacter* sp. (strain FRC-32)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001390;
Q747F5	HEMH_GEOSL	hemH GSU3312	*Geobacter sulfurreducens*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017180;
C6E7U2	HEMH_GEOSM	hemH GM21_0038	*Geobacter* sp. (strain M21)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001661;
C5D6M6	HEMH_GEOSW	hemH GWCH70_0650	*Geobacillus* sp. (strain WCH70)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001638;
A4IKU8	HEMH_GEOTN	hemH GTNG_0570	*Geobacillus thermodenitrificans* (strain NG80-2)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000557;
A5GDG7	HEMH_GEOUR	hemH Gura_0173	*Geobacter uraniireducens* (strain Rf4) (*Geobacter uraniumreducens*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000698;
Q7NMC7	HEMH_GLOVI	hemH gll0839	*Gloeobacter violaceus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000045;
Q5FSJ9	HEMH_GLUOX	hemH GOX0874	*Gluconobacter oxydans* (*Gluconobacter suboxydans*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000009;
Q4QLD6	HEMH_HAEI8	hemH NTHI1329	*Haemophilus influenzae* (strain 86-028NP)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000057;
A5UCU6	HEMH_HAEIE	hemH CGSHiEE_06240	*Haemophilus influenzae* (strain PittEE)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000671;
P43868	HEMH_HAEIN	hemH visA HI_1160	*Haemophilus influenzae* (strain ATCC 51907/DSM 11121/KW20/Rd)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	L42023;
C4K332	HEMH_HAMD5	hemH HDEF_0207	*Hamiltonella defensa* subsp. *Acyrthosiphon pisum* (strain 5AT)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001277;
Q17YG0	HEMH_HELAH	hemH Hac_0490	*Helicobacter acinonychis* (strain Sheeba)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM260522;

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Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
Q7VHH1	HEMH_HELHP	hemH HH_0996	*Helicobacter hepaticus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017125;
B6JMR9	HEMH_HELP2	hemH HPP12_1045	*Helicobacter pylori* (strain P12)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001217;
B5Z875	HEMH_HELPG	hemH HPG27_1021	*Helicobacter pylori* (strain G27)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001173;
Q1CSI9	HEMH_HELPH	hemH HPAG1_1016	*Helicobacter pylori* (strain HPAG1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000241;
Q9ZKD4	HEMH_HELPJ	hemH jhp_1005	*Helicobacter pylori* J99 (*Campylobacter pylori* J99)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE001439;
B2UUI4	HEMH_HELPS	hemH HPSH_05535	*Helicobacter pylori* (strain Shi470)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001072;
P56107	HEMH_HELPY	hemH HP_0376	*Helicobacter pylori* (*Campylobacter pylori*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE000511;
A9B546	HEMH_HERA2	hemH Haur_3547	*Herpetosiphon aurantiacus* (strain ATCC 23779/DSM 785)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000875;
A4G8D5	HEMH_HERAR	hemH HEAR2650	*Herminiimonas arsenicoxydans*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU207211;
P42045	HEMH_HORVU	HEMH	*Hordeum vulgare* (Barley)	4.99.1.1	Ferrochelatase-2, chloroplastic (EC 4.99.1.1) (Ferrochelatase II) (Heme synthase 2) (Protoheme ferro-lyase 2)	D26105; AF020791;
P22830	HEMH_HUMAN	FECH	*Homo sapiens* (Human)	4.99.1.1	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	D00726; AJ250235; BT019958; AK292937; CH471096; BC039841; L36178; AF495859;
Q0BWA5	HEMH_HYPNA	hemH HNE_3567	*Hyphomonas neptunium* (strain ATCC 15444)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000158;
Q5QVZ8	HEMH_IDILO	hemH IL2347	*Idiomarina loihiensis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017340;
A6T228	HEMH_JANMA	hemH mma_2885	*Janthinobacterium* sp. (strain Marseille) (*Minibacterium massiliensis*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000269;
B5Y0N2	HEMH_KLEP3	hemH KPK_4223	*Klebsiella pneumoniae* (strain 342)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000964;
Q036X3	HEMH_LACC3	hemH LSEI_1991	*Lactobacillus casei* (strain ATCC 334)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000423;
B3W963	HEMH_LACCB	hemH LCABL_21650	*Lactobacillus casei* (strain BL23)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	FM177140;
Q9CFB4	HEMH_LACLA	hemH LL1567 L0194	*Lactococcus lactis* subsp. *lactis* (*Streptococcus lactis*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE005176;
A2RJS3	HEMH_LACLM	hemH llmg_0934	*Lactococcus lactis* subsp. *cremoris* (strain MG1363)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM406671;
Q02Y14	HEMH_LACLS	hemH LACR_1661	*Lactococcus lactis* subsp. *cremoris* (strain SK11)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000425;
Q88XC3	HEMH_LACPL	hemH lp_1296	*Lactobacillus plantarum*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL935255;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
A5VLS3	HEMH_LACRD	hemH Lreu_1554	*Lactobacillus reuteri* (strain DSM 20016)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000705;
B2G944	HEMH_LACRJ	hemH LAR_1460	*Lactobacillus reuteri* (strain JCM 1112)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP007281;
Q5X7W3	HEMH_LEGPA	hemH lpp0492	*Legionella pneumophila* (strain Paris)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CR628336;
A5IHG9	HEMH_LEGPC	hemH LPC_2918	*Legionella pneumophila* (strain Corby)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000675;
Q5ZYE7	HEMH_LEGPH	hemH lpg0425	*Legionella pneumophila* subsp. *pneumophila* (strain Philadelphia 1/ ATCC 33152/DSM 7513)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017354;
Q5WZB4	HEMH_LEGPL	hemH lpl0468	*Legionella pneumophila* (strain Lens)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CR628337;
Q6AHF2	HEMH_LEIXX	hemH Lxx01090	*Leifsonia xyli* subsp. *xyli*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE016822;
B0SF60	HEMH_LEPBA	hemH LBF_1123	*Leptospira biflexa* serovar Patoc (strain Patoc 1/Ames)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000777;
Q8GCV0	HEMH_LEPBI	hemH	*Leptospira biflexa*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AY164270;
Q04NU7	HEMH_LEPBJ	hemH LBJ_4018	*Leptospira borgpetersenii* serovar Hardjo-bovis (strain JB197)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000351;
Q04X34	HEMH_LEPBL	hemH LBL_4018	*Leptospira borgpetersenii* serovar Hardjo-bovis (strain L550)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000349;
B0SNJ9	HEMH_LEPBP	hemH LEPBI_I1164	*Leptospira biflexa* serovar Patoc (strain Patoc 1/ATCC 23582/Paris)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000786;
Q03Z41	HEMH_LEUMM	hemH LEUM_0412	*Leuconostoc mesenteroides* subsp. *mesenteroides* (strain ATCC 8293/ NCDO 523)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000414;
Q929G2	HEMH_LISIN	hemH lin2314	*Listeria innocua*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL596171;
Q71XF4	HEMH_LISMF	hemH LMOf2365_2244	*Listeria monocytogenes* serotype 4b (strain F2365)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017262;
Q8Y565	HEMH_LISMO	hemH lmo2211	*Listeria monocytogenes*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL591982;
Q65SV7	HEMH_MANSM	hemH MS1346	*Mannheimia succiniciproducens* (strain MBEL55E)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE016827;
A1U0R2	HEMH_MARAV	hemH Maqu_1496	*Marinobacter aquaeolei* (strain ATCC 700491/DSM 11845/VT8) (*Marinobacter hydrocarbonoclasticus* (strain DSM 11845))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000514;
A6VXF8	HEMH_MARMS	hemH Mmwyl1_2215	*Marinomonas* sp. (strain MWYL1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000749;
Q11ES4	HEMH_MESSB	hemH Meso_2724	*Mesorhizobium* sp. (strain BNC1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000390;
Q607T4	HEMH_METCA	hemH MCA1671	*Methylococcus capsulatus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017282;

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Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
Q1H397	HEMH_METFK	hemH Mfla_0772	*Methylobacillus flagellatus* (strain KT/ ATCC 51484/DSM 6875)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000284;
A2SIR1	HEMH_METPP	hemH Mpe_A2495	*Methylibium petroleiphilum* (strain PM1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000555;
B8EJ84	HEMH_METSB	hemH Msil_3692	*Methylocella silvestris* (strain BL2/ DSM 15510/NCIMB 13906)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001280;
B0JRN7	HEMH_MICAN	hemH MAE_09650	*Microcystis aeruginosa* (strain NIES-843)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP009552;
P22315	HEMH_MOUSE	Fech	*Mus musculus* (Mouse)	4.99.1.1	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	M61215; M59288;
A0QHT7	HEMH_MYCA1	hemH MAV_3293	*Mycobacterium avium* (strain 104)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000479;
B1MC29	HEMH_MYCA9	hemH MAB_2721c	*Mycobacterium abscessus* (strain ATCC 19977/DSM 44196)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU458896;
O07401	HEMH_MYCAV	hemH	*Mycobacterium avium*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AF002133;
P0A577	HEMH_MYCBO	hemH hemZ Mb1521	*Mycobacterium bovis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX248339;
A1KIS5	HEMH_MYCBP	hemH BCG_1547	*Mycobacterium bovis* (strain BCG/ Pasteur 1173P2)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM408590;
C1AND1	HEMH_MYCBT	hemH JTY_1522	*Mycobacterium bovis* (strain BCG/ Tokyo 172/ATCC 35737/TMC 1019)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP010918;
A4T9I1	HEMH_MYCGI	hemH Mflv_3656	*Mycobacterium gilvum* (strain PYR-GCK) (*Mycobacterium flavescens* (strain ATCC 700033/PYR-GCK))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000656;
B8ZS79	HEMH_MYCLB	hemH MLBr01805	*Mycobacterium leprae* (strain Br4923)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	FM211192;
Q9CBM2	HEMH_MYCLE	hemH hemZ ML1805	*Mycobacterium leprae*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL583923;
B2HPD6	HEMH_MYCMM	hemH MMAR_2291	*Mycobacterium marinum* (strain ATCC BAA-535/M)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000854;
Q740Y1	HEMH_MYCPA	hemH MAP_1211	*Mycobacterium paratuberculosis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE016958;
A0QX29	HEMH_MYCS2	hemH MSMEG_3152	*Mycobacterium smegmatis* (strain ATCC 700084/mc(2)155)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000480;
A3PZF2	HEMH_MYCSJ	hemH Mjls_2495	*Mycobacterium* sp. (strain JLS)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000580;
A1UFU2	HEMH_MYCSK	hemH Mkms_2503	*Mycobacterium* sp. (strain KMS)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000518;
Q1B968	HEMH_MYCSS	hemH Mmcs_2458	*Mycobacterium* sp. (strain MCS)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000384;
A5U2I9	HEMH_MYCTA	hemH MRA_1495	*Mycobacterium tuberculosis* (strain ATCC 25177/H37Ra)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000611;
P0A576	HEMH_MYCTU	hemH hemZ Rv1485 MT1532 MTCY277.06	*Mycobacterium tuberculosis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX842576; AE000516;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
A0PNU6	HEMH_MYCUA	hemH MUL_1493	*Mycobacterium ulcerans* (strain Agy99)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000325;
A1T8R1	HEMH_MYCVP	hemH Mvan_2754	*Mycobacterium vanbaalenii* (strain DSM 7251/PYR-1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000511;
Q5F9U6	HEMH_NEIG1	hemH NGO0293	*Neisseria gonorrhoeae* (strain ATCC 700825/FA 1090)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE004969;
Q9JVA5	HEMH_NEIMA	hemH NMA0927	*Neisseria meningitidis* serogroup A	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL157959;
Q9K097	HEMH_NEIMB	hemH NMB0718	*Neisseria meningitidis* serogroup B	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE002098;
A1KSY0	HEMH_NEIMF	hemH NMC0669	*Neisseria meningitidis* serogroup C/ serotype 2a (strain ATCC 700532/ FAM18)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM421808;
Q82UK8	HEMH_NITEU	hemH NE1476	*Nitrosomonas europaea*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL954747;
Q1QI02	HEMH_NITHX	hemH Nham_3415	*Nitrobacter hamburgensis* (strain X14/ DSM 10229)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000319;
Q3JAW7	HEMH_NITOC	hemH Noc_1545	*Nitrosococcus oceani* (strain ATCC 19707/NCIMB 11848)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000127;
A6Q2Y9	HEMH_NITSB	hemH NIS_0734	*Nitratiruptor* sp. (strain SB155-2)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP009178;
Q5YU18	HEMH_NOCFA	hemH NFA_34750	*Nocardia farcinica*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP006618;
B2J9P0	HEMH_NOSP7	hemH Npun_F2510	*Nostoc punctiforme* (strain ATCC 29133/PCC 73102)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001037;
Q8YQR8	HEMH_NOSS1	hemH alr3751	*Nostoc* sp. (strain PCC 7120/UTEX 2576)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000019;
Q8ERX9	HEMH_OCEIH	hemH OB1168	*Oceanobacillus iheyensis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000028;
A6X6W2	HEMH_OCHA4	hemH Oant_4266	*Ochrobactrum anthropi* (strain ATCC 49188/DSM 6882/NCTC 12168)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000759;
B1ZSW3	HEMH_OPITP	hemH Oter_2470	*Opitutus terrae* (strain DSM 11246/ PB90-1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001032;
A2Y3Q5	HEMH_ORYSI	HEMH Osi_018948	*Oryza sativa* subsp. *indica* (Rice)	4.99.1.1	Ferrochelatase-2, chloroplastic (EC 4.99.1.1) (Ferrochelatase II) (Heme synthase 2) (Protoheme ferro-lyase 2)	AB007120;
Q3YA36	HEMH_PANTR	FECH	*Pan troglodytes* (Chimpanzee)	4.99.1.1	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	DQ149645;
Q6MAW8	HEMH_PARUW	hemH pc1557	*Protochlamydia amoebophila* (strain UWE25)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX908798;
P57874	HEMH_PASMU	hemH PM0789	*Pasteurella multocida*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE004439;
A1ASJ7	HEMH_PELPD	hemH Ppro_2715	*Pelobacter propionicus* (strain DSM 2379)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000482;
Q4FNS1	HEMH_PELUB	hemH SAR11_0346	*Pelagibacter ubique*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000084;

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UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
B4RD10	HEMH_PHEZH	hemH PHZ_c3533	*Phenylobacterium zucineum* (strain HLK1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000747;
Q7N0P6	HEMH_PHOLL	hemH plu3835	*Photorhabdus luminescens* subsp. *laumondii*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX571871;
Q6LTE0	HEMH_PHOPR	hemH PBPRA1025	*Photobacterium profundum* (*Photobacterium* sp. (strain SS9))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CR378666;
Q12BZ9	HEMH_POLSJ	hemH Bpro_2013	*Polaromonas* sp. (strain JS666/ATCC BAA-500)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000316;
B2RHB4	HEMH_PORG3	hemH PGN_0240	*Porphyromonas gingivalis* (strain ATCC 33277/DSM 20709/JCM 12257)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP009380;
Q7MXP4	HEMH_PORGI	hemH PG_0127	*Porphyromonas gingivalis* (*Bacteroides gingivalis*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE015924;
P72183	HEMH_PROFF	hemH	*Propionibacterium freudenreichii* subsp. *freudenreichii*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	D85417; U51164;
A3PBP9	HEMH_PROM0	hemH P9301_05511	*Prochlorococcus marinus* (strain MIT 9301)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000576;
A2C0Y4	HEMH_PROM1	hemH NATL1_05821	*Prochlorococcus marinus* (strain NATL1A)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000553;
A8G3P0	HEMH_PROM2	hemH P9215_06061	*Prochlorococcus marinus* (strain MIT 9215)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000825;
A2C7Q7	HEMH_PROM3	hemH P9303_07661	*Prochlorococcus marinus* (strain MIT 9303)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000554;
A9BEE9	HEMH_PROM4	hemH P9211_05281	*Prochlorococcus marinus* (strain MIT 9211)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000878;
A2BVI7	HEMH_PROM5	hemH P9515_05891	*Prochlorococcus marinus* (strain MIT 9515)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000552;
Q31C09	HEMH_PROM9	hemH PMT9312_0525	*Prochlorococcus marinus* (strain MIT 9312)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000111;
Q7VD58	HEMH_PROMA	hemH Pro_0525	*Prochlorococcus marinus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017126;
B4F1Q1	HEMH_PROMH	hemH PMI2183	*Proteus mirabilis* (strain HI4320)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM942759;
Q7V6C6	HEMH_PROMM	hemH PMT_1240	*Prochlorococcus marinus* (strain MIT 9313)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX548175;
Q7V2F5	HEMH_PROMP	hemH PMM0525	*Prochlorococcus marinus* subsp. *pastoris* (strain CCMP1986/MED4)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX548174;
A2BQ06	HEMH_PROMS	hemH A9601_05811	*Prochlorococcus marinus* (strain AS9601)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000551;
Q46GQ1	HEMH_PROMT	hemH PMN2A_1857	*Prochlorococcus marinus* (strain NATL2A)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000095;
Q48MT5	HEMH_PSE14	hemH PSPPH_1016	*Pseudomonas syringae* pv. phaseolicola (strain 1448A/Race 6)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000058;
Q15NQ1	HEMH_PSEA6	hemH Patl_3987	*Pseudoalteromonas atlantica* (strain T6c/BAA-1087)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000388;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
A6VC51	HEMH_PSEA7	hemH PSPA7_5304	*Pseudomonas aeruginosa* (strain PA7)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000744;
B7V0K1	HEMH_PSEA8	hemH PLES_50411	*Pseudomonas aeruginosa* (strain LESB58)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	FM209186;
Q02G19	HEMH_PSEAB	hemH PA14_61580	*Pseudomonas aeruginosa* (strain UCBPP-PA14)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000438;
Q9HVD7	HEMH_PSEAE	hemH PA4655	*Pseudomonas aeruginosa*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE004091;
Q1IEW1	HEMH_PSEE4	hemH PSEEN0886	*Pseudomonas entomophila* (strain L48)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CT573326;
Q4K6B3	HEMH_PSEF5	hemH PFL_5141	*Pseudomonas fluorescens* (strain Pf-5/ ATCC BAA-477)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000076;
P57778	HEMH_PSEFC	hemH	*Pseudomonas fluorescens* biotype C	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AF314196;
C3KDN7	HEMH_PSEFS	hemH PFLU_0756	*Pseudomonas fluorescens* (strain SBW25)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM181176;
A4XR78	HEMH_PSEMY	hemH Pmen_1077	*Pseudomonas mendocina* (strain ymp)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000680;
A5VYH6	HEMH_PSEP1	hemH Pput_0772	*Pseudomonas putida* (strain F1/ ATCC 700007)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000712;
Q3K6Y7	HEMH_PSEPF	hemH Pfl01_4730	*Pseudomonas fluorescens* (strain Pf0-1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000094;
B0KNF5	HEMH_PSEPG	hemH PputGB1_0785	*Pseudomonas putida* (strain GB-1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000926;
Q88PV4	HEMH_PSEPK	hemH PP_0744	*Pseudomonas putida* (strain KT2440)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE015451;
B1JER5	HEMH_PSEPW	hemH PputW619_4445	*Pseudomonas putida* (strain W619)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000949;
Q888A2	HEMH_PSESM	hemH PSPTO_1128	*Pseudomonas syringae* pv. tomato	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE016853;
Q4ZXU9	HEMH_PSEU2	hemH Psyr_0967	*Pseudomonas syringae* pv. *syringae* (strain B728a)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000075;
A4VKZ1	HEMH_PSEU5	hemH PST_1968	*Pseudomonas stutzeri* (strain A1501)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000304;
Q4FUR3	HEMH_PSYA2	hemH Psyc_0376	*Psychrobacter arcticus* (strain DSM 17307/273-4)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000082;
Q1QDQ5	HEMH_PSYCK	hemH Pcryo_0415	*Psychrobacter cryohalolentis* (strain K5)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000323;
A5WH56	HEMH_PSYWF	hemH PsycPRwf_2057	*Psychrobacter* sp. (strain PRwf-1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000713;
Q1LPN9	HEMH_RALME	hemH Rmet_1001	*Ralstonia metallidurans* (strain CH34/ ATCC 43123/DSM 2839)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000352;
B2UBQ0	HEMH_RALPJ	hemH Rpic_2877	*Ralstonia pickettii* (strain 12J)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001068;
Q8XW32	HEMH_RALSO	hemH RSc2643 RS04573	*Ralstonia solanacearum* (*Pseudomonas solanacearum*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL646052;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
B3PZU8	HEMH_RHIE6	hemH RHECIAT_CH0003810	*Rhizobium etli* (strain CIAT 652)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001074;
Q2K4C4	HEMH_RHIEC	hemH RHE_CH03556	*Rhizobium etli* (strain CFN 42/ATCC 51251)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000133;
Q1MBW8	HEMH_RHIL3	hemH RL4076	*Rhizobium leguminosarum* bv. viciae (strain 3841)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM236080;
Q98H61	HEMH_RHILO	hemH mir3019	*Rhizobium loti* (*Mesorhizobium loti*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000012;
B5ZPG3	HEMH_RHILW	hemH Rleg2_3304	*Rhizobium leguminosarum* bv. trifolii (strain WSM2304)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001191;
Q92M52	HEMH_RHIME	hemH R02803 SMc04019	*Rhizobium meliloti* (*Sinorhizobium meliloti*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL591688; AF461430;
Q7UFZ7	HEMH_RHOBA	hemH RB8233	*Rhodopirellula baltica*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX294147;
Q59735	HEMH_RHOCA	hemH	*Rhodobacter capsulatus* (*Rhodopseudomonas capsulata*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	U34391;
Q2IRD5	HEMH_RHOP2	hemH RPB_4542	*Rhodopseudomonas palustris* (strain HaA2)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000250;
Q07H58	HEMH_RHOP5	hemH RPE_4807	*Rhodopseudomonas palustris* (strain BisA53)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000463;
Q6NBF3	HEMH_RHOPA	hemH RPA0875	*Rhodopseudomonas palustris*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX572595;
Q13CU0	HEMH_RHOPS	hemH RPD_0861	*Rhodopseudomonas palustris* (strain BisB5)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000283;
B3QF66	HEMH_RHOPT	hemH Rpal_0943	*Rhodopseudomonas palustris* (strain TIE-1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001096;
A8GYD7	HEMH_RICB8	hemH A1I_07945	*Rickettsia bellii* (strain OSU 85-389)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000849;
Q1RGK5	HEMH_RICBR	hemH RBE_1428	*Rickettsia bellii* (strain RML369-C)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000087;
A8F0B2	HEMH_RICCK	hemH A1E_05670	*Rickettsia canadensis* (strain McKiel)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000409;
Q92FV4	HEMH_RICCN	hemH RC1373	*Rickettsia conorii*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE006914;
Q4UJN9	HEMH_RICFE	hemH RF_1399	*Rickettsia felis* (*Rickettsia azadi*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000053;
Q9ZC84	HEMH_RICPR	hemH RP884	*Rickettsia prowazekii*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AJ235273;
Q68VM9	HEMH_RICTY	hemH RT0876	*Rickettsia typhi*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017197;
B5EXN2	HEMH_SALA4	hemH SeAg_B0535	*Salmonella agona* (strain SL483)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001138;
A9MLY4	HEMH_SALAR	hemH SARI_02445	*Salmonella arizonae* (strain ATCC BAA-731/CDC346-86/RSK2980)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000880;
Q57S74	HEMH_SALCH	hemH SCH_0531	*Salmonella choleraesuis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017220;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
B5FLJ9	HEMH_SALDC	hemH SeD_A0536	*Salmonella dublin* (strain CT_02021853)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001144;
B5QU78	HEMH_SALEP	hemH SEN0470	*Salmonella enteritidis* PT4 (strain P125109)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM933172;
B5R613	HEMH_SALG2	hemH SG0500	*Salmonella gallinarum* (strain 287/91/ NCTC 13346)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM933173;
B4T9I3	HEMH_SALHS	hemH SeHA_C0595	*Salmonella heidelberg* (strain SL476)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001120;
B4SWY3	HEMH_SALNS	hemH SNSL254_A0541	*Salmonella newport* (strain SL254)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001113;
Q5PFJ1	HEMH_SALPA	hemH SPA2233	*Salmonella paratyphi* A	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000026;
A9MW82	HEMH_SALPB	hemH SPAB_03079	*Salmonella paratyphi* B (strain ATCC BAA-1250/SPB7)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000886;
C0PVE1	HEMH_SALPC	hemH SPC_0504	*Salmonella paratyphi* C (strain RKS4594)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000857;
B5BD43	HEMH_SALPK	hemH SSPA2076	*Salmonella paratyphi* A (strain AKU_12601)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	FM200053;
B4TMG7	HEMH_SALSV	hemH SeSA_A0551	*Salmonella schwarzengrund* (strain CVM19633)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001127;
Q8Z8T2	HEMH_SALTI	hemH STY0533 t2371	*Salmonella typhi*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL627267; AE014613;
P37408	HEMH_SALTY	hemH visA STM0489	*Salmonella typhimurium*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE006468; L26246;
O59786	HEMH_SCHPO	SPCC320.09	*Schizosaccharomyces pombe* (strain ATCC 38366/972) (Fission yeast)	4.99.1.1	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CU329672;
A8GAV7	HEMH_SERP5	hemH Spro_1143	*Serratia proteamaculans* (strain 568)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000826;
Q12KR9	HEMH_SHEDO	hemH Sden_2678	*Shewanella denitrificans* (strain OS217/ ATCC BAA-1090/DSM 15013)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000302;
B2U4S8	HEMH_SHIB3	hemH SbBS512_E0408	*Shigella boydii* serotype 18 (strain CDC 3083-94/BS512)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001063;
Q325C1	HEMH_SHIBS	hemH SBO_0375	*Shigella boydii* serotype 4 (strain Sb227)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000036;
Q32J55	HEMH_SHIDS	hemH SDY_0444	*Shigella dysenteriae* serotype 1 (strain Sd197)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000034;
Q0T7B0	HEMH_SHIF8	hemH SFV_0448	*Shigella flexneri* serotype 5b (strain 8401)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000266;
Q83SE5	HEMH_SHIFL	hemH SF0420 S0427	*Shigella flexneri*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE005674; AE014073;
Q3Z4S4	HEMH_SHISS	hemH SSON_0462	*Shigella sonnei* (strain Ss046)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000038;
Q2NV56	HEMH_SODGM	hemH SG0694	*Sodalis glossinidius* (strain morsitans)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP008232;
Q5HEU3	HEMH_STAAC	hemH SACOL1888	*Staphylococcus aureus* (strain COL)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000046;

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UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
P64124	HEMH_STAAM	hemH SAV1833	*Staphylococcus aureus* (strain Mu50/ ATCC 700699)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000017;
P64125	HEMH_STAAN	hemH SA1651	*Staphylococcus aureus* (strain N315)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000018;
Q6GFM4	HEMH_STAAR	hemH hemF SAR1924	*Staphylococcus aureus* (strain MRSA252)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX571856;
Q6G8A3	HEMH_STAAS	hemH SAS1754	*Staphylococcus aureus* (strain MSSA476)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX571857;
P64126	HEMH_STAAW	hemH MW1773	*Staphylococcus aureus* (strain MW2)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000033;
Q5HNA5	HEMH_STAEQ	hemH SERP1367	*Staphylococcus epidermidis* (strain ATCC 35984/RP62A)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000029;
Q8CNS1	HEMH_STAES	hemH SE_1512	*Staphylococcus epidermidis* (strain ATCC 12228)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE015929;
Q49YM6	HEMH_STAS1	hemH SSP0966	*Staphylococcus saprophyticus* subsp. *saprophyticus* (strain ATCC 15305/ DSM 20229)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP008934;
Q82KJ6	HEMH_STRAW	hemH SAV_2407	*Streptomyces avermitilis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000030;
O50533	HEMH_STRCO	hemH SCO5859 SC9B10.26	*Streptomyces coelicolor*	4.99.1.1	Probable ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL939125;
B1VXN6	HEMH_STRGG	hemH SGR_1673	*Streptomyces griseus* subsp. *griseus* (strain JCM 4626/NBRC 13350)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP009493;
B4SNS0	HEMH_STRM5	hemH Smal_3991	*Stenotrophomonas maltophilia* (strain R551-3)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001111;
Q8CWW4	HEMH_STRMU	hemH hemZ SMU_2063	*Streptococcus mutans*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE014133;
Q04KS4	HEMH_STRP2	hemH SPD_0895	*Streptococcus pneumoniae* serotype 2 (strain D39/NCTC 7466)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000410:
C1C6Y5	HEMH_STRP7	hemH SP70585_1048	*Streptococcus pneumoniae* (strain 70585)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000918;
B1IBG8	HEMH_STRPI	hemH SPH_1111	*Streptococcus pneumoniae* (strain Hungary19A-6)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000936;
B8ZPG2	HEMH_STRPJ	hemH SPN23F09340	*Streptococcus pneumoniae* (strain ATCC 700669/Spain 23F-1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	FM211187;
Q97R30	HEMH_STRPN	hemH SP_1009	*Streptococcus pneumoniae*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE005672;
B2IPG3	HEMH_STRPS	hemH SPCG_0985	*Streptococcus pneumoniae* (strain CGSP14)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001033;
Q8DQ04	HEMH_STRR6	hemH spr0914	*Streptococcus pneumoniae* (strain ATCC BAA-255/R6)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE007317;
C1CE03	HEMH_STRZJ	hemH SPJ_0949	*Streptococcus pneumoniae* (strain JJA)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000919;
C1CRD1	HEMH_STRZT	hemH SPT_1062	*Streptococcus pneumoniae* (strain Taiwan19F-14)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000921;

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Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
Q30S35	HEMH_SULDN	hemH Suden_0918	*Sulfurimonas denitrificans* (strain ATCC 33889/DSM 1251) (*Thiomicrospira denitrificans* (strain ATCC 33889/DSM 1251))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000153;
B2V955	HEMH_SULSY	hemH SYO3AOP1_0845	*Sulfurihydrogenibium* sp. (strain YO3AOP1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001080;
Q67T48	HEMH_SYMTH	hemH STH160	*Symbiobacterium thermophilum*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP006840;
Q31S00	HEMH_SYNE7	hemH Synpcc7942_0137	*Synechococcus elongatus* (strain PCC 7942) (*Anacystis nidulans* R2)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000100;
Q2JVK5	HEMH_SYNJA	hemH CYA_1034	*Synechococcus* sp. (strain JA-3-3Ab) (*Cyanobacteria bacterium* Yellowstone A-Prime)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000239;
Q2JHZ4	HEMH_SYNJB	hemH CYB_2857	*Synechococcus* sp. (strain JA-2-3B'a(2-13)) (*Cyanobacteria bacterium* Yellowstone B-Prime)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000240;
B1XL79	HEMH_SYNP2	hemH SYNPCC7002_A2589	*Synechococcus* sp. (strain ATCC 27264/PCC 7002/PR-6) (*Agmenellum quadruplicatum*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000951;
Q5N2B2	HEMH_SYNP6	hemH syc1368_c	*Synechococcus* sp. (strain ATCC 27144/PCC 6301/SAUG 1402/1) (*Anacystis nidulans*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AP008231;
A5GJF5	HEMH_SYNPW	hemH SynWH7803_0644	*Synechococcus* sp. (strain WH7803)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CT971583;
Q7U5G0	HEMH_SYNPX	hemH SYNW1747	*Synechococcus* sp. (strain WH8102)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX569693;
A5GS98	HEMH_SYNR3	hemH SynRCC307_0854	*Synechococcus* sp. (strain RCC307)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CT978603;
Q0I8L9	HEMH_SYNS3	hemH sync_2000	*Synechococcus* sp. (strain CC9311)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000435;
Q3ALP2	HEMH_SYNSC	hemH Syncc9605_0716	*Synechococcus* sp. (strain CC9605)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000110;
P54225	HEMH_SYNY3	hemH slr0839	*Synechocystis* sp. (strain ATCC 27184/ PCC 6803/N-1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000022;
C5BIF4	HEMH_TERTT	hemH TERTU_4338	*Teredinibacter turnerae* (strain ATCC 39867/T7901)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001614;
Q9HLB8	HEMH_THEAC	hemH Ta0311	*Thermoplasma acidophilum* (strain ATCC 25905/DSM 1728/JCM 9062/ NBRC 15155/AMRC-C165)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL445063;
Q8DGU6	HEMH_THEEB	hemH tlr2216	*Thermosynechococcus elongatus* (strain BP-1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000039;
Q72L32	HEMH_THET2	hemH TT_C0231	*Thermus thermophilus* (strain HB27/ ATCC BAA-163/DSM 7039)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017221;
Q978U9	HEMH_THEVO	hemH TV1316 TVG1357926	*Thermoplasma volcanium* (strain ATCC 51530/DSM 4299/IFO 15438/ JCM 9571/GSS1)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000011;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
Q31EG9	HEMH_THICR	hemH Tcr_1864	*Thiomicrospira crunogena* (strain XCL-2)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000109;
Q3SHA3	HEMH_THIDA	hemH Tbd_2033	*Thiobacillus denitrificans* (strain ATCC 25259)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000116;
B8GU82	HEMH_THISH	hemH Tgr7_0266	*Thioalkalivibrio* sp. (strain HL-EbGR7)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001339;
Q10WR6	HEMH_TRIEI	hemH Tery_4313	*Trichodesmium erythraeum* (strain IMS101)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000393;
Q83H94	HEMH_TROW8	hemH TW747	*Tropheryma whipplei* (strain TW08/27) (Whipple's *bacillus*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX251412;
Q83FJ2	HEMH_TROWT	hemH TWT_733	*Tropheryma whipplei* (strain Twist) (Whipple's *bacillus*)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE014184;
Q9KTB6	HEMH_VIBCH	hemH VC_0987	*Vibrio cholerae*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE003852;
Q5E6Q7	HEMH_VIBF1	hemH VF_0794	*Vibrio fischeri* (strain ATCC 700601/ ES114)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000020;
B5FBZ6	HEMH_VIBFM	hemH VFMJ11_0831	*Vibrio fischeri* (strain MJ11)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001139;
Q87RH3	HEMH_VIBPA	hemH VP0823	*Vibrio parahaemolyticus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000031;
B7VII4	HEMH_VIBSL	hemH VS_2267	*Vibrio splendidus* (strain LGP32) (*Vibrio splendidus* (strain Mel32))	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	FM954972;
Q8DFM2	HEMH_VIBVU	hemH VV1_0187	*Vibrio vulnificus*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE016795;
Q7MMR4	HEMH_VIBVY	hemH VV1003	*Vibrio vulnificus* (strain YJ016)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000037;
Q8D226	HEMH_WIGBR	hemH WIGBR5290	*Wigglesworthia glossinidia brevipalpis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BA000021;
Q73FY6	HEMH_WOLPM	hemH WD_1186	*Wolbachia pipientis* wMel	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017196;
B3CLU1	HEMH_WOLPP	hemH WP0751	*Wolbachia pipientis* subsp. *Culex pipiens* (strain wPip)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM999887;
Q7M7P9	HEMH_WOLSU	hemH WS2157	*Wolinella succinogenes*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX571662;
Q5GRR7	HEMH_WOLTR	hemH Wbm0719	*Wolbachia* sp. subsp. *Brugia malayi* (strain TRS)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE017321;
C0R4L0	HEMH_WOLWR	hemH WRi_011580	*Wolbachia* sp. subsp. *Drosophila simulans* (strain wRi)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001391;
Q8PEX0	HEMH_XANAC	hemH XAC4220	*Xanthomonas axonopodis* pv. citri (Citrus canker)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE008923;
Q8P3H6	HEMH_XANCP	hemH XCC4095	*Xanthomonas campestris* pv. *campestris*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE008922;
O57478	HEMH_XENLA	fech	*Xenopus laevis* (African clawed frog)	4.99.1.1	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AF036617;
B2I7H7	HEMH_XYLF2	hemH XfasM23_1664	*Xylella fastidiosa* (strain M23)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001011;

TABLE 3-continued

Characterized Ferrochelatase Enzymes

UniProKB/ SwissProt Accession No.	Entry Name	Gene Names	Organism	EC Number	Protein Names	EMBL Accession No.
Q9PFU1	HEMH_XYLFA	hemH XF_0566	*Xylella fastidiosa*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE003849;
B0U456	HEMH_XYLFM	hemH Xfasm12_1736	*Xylella fastidiosa* (strain M12)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000941;
Q87B82	HEMH_XYLFT	hemH PD_1576	*Xylella fastidiosa* (strain Temecula1/ ATCC 700964)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AE009442;
P16622	HEMH_YEAST	HEM15 YOR176W	*Saccharomyces cerevisiae* (strain ATCC 204508/S288c) (Baker's yeast)	4.99.1.1	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	J05395; X54514; Z75084; BK006948;
A1JNA4	HEMH_YERE8	hemH YE3088	*Yersinia enterocolitica* serotype O:8/ biotype 1B (strain 8081)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AM286415;
P43413	HEMH_YEREN	hemH	*Yersinia enterocolitica*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	Z47767; U46859;
A7FL85	HEMH_YERP3	hemH YpsIP31758_3053	*Yersinia pseudotuberculosis* serotype O:1b (strain IP 31758)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000720;
Q1C4Q0	HEMH_YERPA	hemH YPA_2610	*Yersinia pestis* bv. Antiqua (strain Antiqua)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000308;
B2K6Z7	HEMH_YERPB	hemH YPTS_1040	*Yersinia pseudotuberculosis* serotype IB (strain PB1/+)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP001048;
Q8ZC98	HEMH_YERPE	hemH YPO3117 y1066 YP_0813	*Yersinia pestis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AL590842; AE009952; AE017042;
A9R0Q8	HEMH_YERPG	hemH YpAngola_A2895	*Yersinia pestis* bv. Antiqua (strain Angola)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000901;
Q1CL26	HEMH_YERPN	hemH YPN_0972 YP516_1054	*Yersinia pestis* bv. Antiqua (strain Nepal516)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000305; ACNQ01000008;
A4TPA3	HEMH_YERPP	hemH YPDSF_2753	*Yersinia pestis* (strain Pestoides F)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000668;
Q05338	HEMH_YERPS	hemH YPTB0997	*Yersinia pseudotuberculosis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	BX936398; AF461770;
B1JHN0	HEMH_YERPY	hemH YPK_3193	*Yersinia pseudotuberculosis* serotype O:3 (strain YPIII)	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	CP000950;
P57779	HEMH_ZYMMO	hemH ZMO0303	*Zymomonas mobilis*	4.99.1.1	Ferrochelatase (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	AF212041; AE008692;

[0027] Suitable ferrochelatases of the present invention can also be identified by homology or sequence identity to the ferrochelatases disclosed in Table 3. Suitable ferrochelatases include those having an amino acid or nucleotide sequence identity of at least about 25 percent, more preferably at least about 30 to 40 percent, more preferably at least 50 to 60 percent, more preferably at least about 70 to 80 percent, most preferably at least about 85 to 95 percent as compared to a characterized ferrochelatase sequence of Table 3.

[0028] Mutant or modified ferrochelatase enzymes that retain their enzymatic activity (i.e., capable of catalyzing the insertion of ferrous iron into protoporphyrin IX to form protoheme (EC 4.99.1.1)) are also suitable for use in the present invention. Modifications or mutations to known ferrochelatase nucleotide sequences, such as nucleotide deletions, insertions, or substitutions that do not alter the coding sequence of the active region of the ferrochelatase enzyme or do not alter the activity of the encoded ferrochelatase enzyme are suitable for use in the methods of the present invention. For example, eukaryotic ferrochelatases possess an amino-terminus signal sequence that is lacking in prokaryote ferrochelatases (Dailey et al., "Ferrochelatase at the Millennium: Structures, Mechanisms, and [2Fe-2S] Clusters," *Cell. Mol. Life. Sci.* 57:1909-1926 (2000) which is hereby incorporated by reference in its entirety). Therefore, recombinant eukaryotic ferrochelatase sequences containing modifications or deletions to this portion of the encoded protein that do not alter enzyme activity are suitable for use in the present invention. Likewise, eukaryotic ferrochelatases also possess a carboxyl-terminal extension sequence that is lacking in a majority of prokaryotic ferrochelatases (Dailey et al., "Ferrochelatase at the Millennium: Structures, Mechanisms, and [2Fe-2S] Clusters," *Cell. Mol. Life. Sci.* 57:1909-1926 (2000), which is hereby incorporated by reference in its entirety). Recombinant eukaryotic ferrochelatase sequences containing modifications or deletions to this extension region are also suitable for use in the methods of the present invention. Similarly, recombinant ferrochelatase sequences containing single-base mutations that do not alter the conserved amino acid residues of the ferrochelatase protein acids (Dailey et al., "Ferrochelatase at the Millennium: Structures, Mechanisms, and [2Fe-2S] Clusters," *Cell. Mol. Life. Sci.* 57:1909-1926 (2000), which is hereby incorporated by reference in its entirety) or the enzyme active site are also suitable for use in the present invention.

[0029] In accordance with this aspect of the invention, co-expression of the recombinant heme-binding protein and ferrochelatase protein, or polypeptide thereof, is carried out in the presence of one or more heme precursors, Suitable heme precursors include, without limitation, δ-amino levulinic acid, succinyl CoA, glycine, glutamate, glutamate-1 semialdehyde, porphobilinogen, hydroxymethylbilane, and protoporphyrin.

[0030] Co-expression of the recombinant heme-binding protein and ferrochelatase protein or polypeptide can be carried out in any one of the commonly known systems that are available in the art for heterologous protein expression, including, without limitation, eukaryotic and prokaryotic expression systems, and cell-free translation systems as described herein.

[0031] Techniques and protocols for manipulation of nucleic acids, including, for example, the preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Current Protocols in Molecular Biology*, Ausubel et al. eds., (1992), which is hereby incorporated by reference in its entirety.

[0032] Typically, a nucleic acid molecule encoding all or part of a protein of interest, i.e., a heme-binding protein, is obtained using methods such as those described herein. The protein-encoding nucleic acid sequence is cloned into an expression vector that is suitable for the particular host cell of interest using standard recombinant DNA procedures. Suitable expression vectors include those which contain replicon and control sequences that are derived from species compatible with the host cell.

[0033] Expression vectors include (among other elements) regulatory sequences (e.g., promoters) that can be operably linked to the desired protein-encoding nucleic acid molecule to cause the expression of such nucleic acid molecule in the host cell. Together, the regulatory sequences and the protein-encoding nucleic acid sequence are an expression construct. Expression vectors may also include an origin of replication, marker genes that provide phenotypic selection in transformed cells, one or more other promoters, and a polylinker region containing several restriction sites for insertion of heterologous nucleic acid sequences.

[0034] Expression vectors useful for expression of heterologous protein(s) in a multitude of host cells are well known in the art (Sambrook and Russell, *Molecular Cloning: a Laboratory Manual* 3rd ed. (2001), which is hereby incorporated by reference in its entirety), and some specific examples are provided herein. The host cell is transfected with (or infected with a virus containing) the expression vector using any method suitable for the particular host cell. Such transfection methods are also well known in the art and non-limiting exemplary methods are described herein. The transfected (also called, transformed) host cell is capable of expressing the protein encoded by the corresponding nucleic acid sequence in the expression construct. Transient or stable transfection of the host cell with one or more expression vectors is contemplated by the present disclosure.

[0035] In one embodiment of the present invention, the nucleotide sequence encoding the desired heme-binding protein is inserted into one expression vector and the nucleotide sequence encoding the ferrochelatase is inserted into a second expression vector. In this embodiment, the two expression vectors are co-transfected into an appropriate host cell for transcription and translation. In another embodiment of the invention, both nucleotide sequences are inserted into one expression vector and the single expression vector encodes both the recombinant heme-binding protein and the recombinant ferrochelatase.

[0036] Many different types of cells may be used to express heterologous proteins, such as bacterial, archaeal, yeast, fungal, insect, vertebrate (such as mammalian cells), and plant cells, including primary cells and immortal cell lines. Numerous representatives of each cell type are commonly used and are available from a wide variety of commercial sources, including, for example, the American Tissue Culture Collection (ATCC). Further details of some specific embodiments are discussed below.

[0037] Prokaryotes, such as bacteria, may be used as host cells. Prokaryotic expression systems are advantageous, at least, because of culture affordability, ease of genetic manipulation, and high yields of desired product(s). As described herein, *E. coli* BL21 (DE3) is a suitable prokaryotic host cell. Other suitable *E. coli* host cells include, without limitation, *E.*

coli K12 strain 94 (ATCC No. 31,446), coli strain W3 110 (ATCC No, 27,325), *E. coli* X1776 (ATCC No, 31,537), *E. coli* B, and many other strains, such as HB101, JM101, NM522, NM538, NM539, B1-21, B1-21 (DE3) pLysS, Origami B, OmpT-defective CD41, CD43 (DE3), and phosphatidylenthanolamine (PE)-deficient AD93. Similarly, other species and genera of prokaryotes including *Pseudomonas aeruginosa, Salmonella gastroenteritis* (*typhimirium*), *S. typhi, S. enteriditis, Shigella flexneri, S. sonnie, S. dysenteriae, Neisseria gonorrhoeae, N. meningitides, Haemophilus influenzae, H. pleuropneumoniae, Pasteurella haemolytica, P. multilocida, Legionella pneumophila, Treponema pallidum, T. denticola, T. orales, Borrelia burgdorferi, Borrelia* spp., *Leptospira interrogans, Klebsiella pneumoniae, Proteus vulgaris, P. morganii, P. mirabilis, Rickettsia prowazeki, R. typhi, R. richettsii, Porphyromonas* (*Bacteriodes*) *gingivalis, Chlamydia psittaci, C. pneumoniae, C. trachomatis, Campylobacter jejuni, C. intermedis, C. fetus, Helicobacter pylori, Francisella tularenisis, Vibrio cholerae, Vibrio parahaemolyticus, Bordetella pertussis, Burkholderie pseudomallei, Brucella abortus, B. susi, B. melitensis, B. canis, Spirillum minus, Pseudomonas mallei, Aeromonas hydrophila, A. salmonicida, Lactococcus lactis*, and *Yersinia pestis*, may all be used as prokaryotic expression hosts.

[0038] Prokaryotic host cells or other host cells with rigid cell walls may be transformed using any method known in the art, including, for example, calcium phosphate precipitation, or electroporation. Representative prokaryote transformation techniques are described in Hanahan et al., "Plasmid Transformation of *Escherichia coli* and Other Bacteria,"*Meth. Enzymol.,* 204:63-113 (1991), which is hereby incorporated by reference in its entirety.

[0039] Vectors typically used for transformation of *E. coli* include, without limitation, pBR322, pUC18, pUC19, pUC118, pUC119, Bluescript M13 and derivatives thereof. Numerous such plasmids are commercially available and are well known in the art.

[0040] Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA ("mRNA") translation). Transcription of DNA is dependent upon the presence of a promoter, which is a DNA sequence that directs the binding of RNA polymerase, and thereby promotes mRNA synthesis. Promoters vary in their strength (i.e., their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters to obtain a high level of transcription and, hence, expression. Therefore, depending upon the host system utilized, any one of a number of suitable promoters may also be incorporated into the expression vector carrying the nucleic acid molecule(s) of the present invention. For instance, when using *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, lac promoter, trp promoter, recA promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to lacUV5, ompF, lpp, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid trp-lacUV5 (tac) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

[0041] Translation of mRNA in prokaryotes depends upon the presence of the proper prokaryotic signals, which differ from those of eukaryotes. Efficient translation of mRNA in prokaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, *Methods in Enzymology,* 68:473 (1979), which is hereby incorporated by reference in its entirety.

[0042] Recombinant heme-binding proteins can also be produced in archaeal expression systems. Species that are suitable for serving as hosts include, without limitation, *Methanosarcina acetivorans* and *Sulfolobus solfataricus* (see e.g., Albers et al., "Production of Recombinant and Tagged Proteins in the Hyerthermophilic Archaeon *Sulfolobus solfataricus," Appl. Environ. Microbial.* 72(1):102-11 (2006) and Jonuscheit et al., "A Reporter Gene System for the Hyperthermophilic Archaean *Sulfolobus solfataricus* based on a Selectable and Integrative Shuttle Vector,"*Mol. Microbiol.* 48(5):1241-52 (2003), which is hereby incorporated by reference in its entirety).

[0043] Fungal protein expression systems can also be utilized in the methods of the present invention to efficiently produce a functional recombinant heme-binding protein. Fungal species are considered safer than animal cells because they pose little risk of contamination by viruses, prions, or endotoxins. Additionally, fungal systems are more efficient and economical than mammalian expression systems. There are several fungal species which have been used as hosts for the expression of recombinant proteins, including, without limitation, yeast (e.g., *Pichia pastoris, Kluyveromyces lactis*, and *Saccharomyces cerevisiae*), soil fungus (e.g., *Trichoderma reesei*), and black mould fungus (e.g., *Aspergillus niger*).

[0044] Yeast strains and yeast-derived vectors are used commonly for the expression of heterologous proteins. For instance, *Pichia pastoris* expression systems, may be used to co-express a recombinant heme-binding protein of interest with ferrochelatase (see e.g., Ballew N., "Revolutionizing Protein Production in Fungi," *Innovations in Pharmaceutical Technology* 70-76 (June 2004), which is hereby incorporated by reference in its entirety). Such systems include suitable *Pichia pastoris* strains, vectors, reagents, transformants, sequencing primers, and media. Available strains include KM71H (a prototrophic strain), SMD1168H (a prototrophic strain), and SMD1168 (a pep4 mutant strain) (Invitrogen).

[0045] *Saccharomyces cerevisiae* is also commonly used in heterologous expression systems. The plasmid YRp7 is commonly used as an expression vector in *Saccharomyces* (Stinchcomb et al., "Isolation and Characterization of a Yeast Chromosomal Replicator," *Nature* 282:39-43 (1979); Kingsman et al., "Replication in *Saccharomyces Cerevisiae* of Plasmid pBR313 Carrying DNA from the Yeast trp1 Region," *Gene* 7:141-152 (1979); and Tschemper et al., "Sequence of a Yeast DNA Fragment Containing a Chromosomal Replicator and the TRP1 Gene," *Gene* 10:157-166 (1980), which are hereby incorporated by reference in their entirety). This plasmid contains the trp1 gene that provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, such as strains ATCC No. 44,076 and PEP4-1 (Jones, E. W., "Proteinase Mutants of *Saccharomyces cerevisiae," Genetics* 85:23-33 (1977), which is hereby incorporated by reference in its entirety). The presence of the trp1

lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

[0046] Yeast host cells can be transformed using the polyethylene glycol method, as described by Hinnen (Hinnen et al., "Transformation of Yeast," *Proc. Natl. Acad. Sci. U.S.A.* 75:1929-1933 (1978), which is hereby incorporated by reference in its entirety). Additional yeast transformation protocols are set forth in Gietz et al., "Improved Method for High Efficiency Transformation of Intact Yeast Cells," *Nucl. Acids Res.* 20(6):1425 (1992)) and Reeves et al. "A Yeast Intron as a Translation Terminator in a Plasmid Shuttle Vector," *Yeast Res.* 4(6):573-597 (2004), which are hereby incorporated by reference in their entirety.

[0047] Suitable promoting sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase (Hitzeman et al., "Isolation and Characterization of the Yeast 3-Phosphoglycerokinase Gene (PGK) by an Immunological Screening Technique," *J. Biol. Chem.* 255:12073-12080 (1980), which is hereby incorporated by reference in its entirety) or other glycolytic enzymes (Hess et al., "Cooperation of Glycolytic Enzymes," *J. Adv. Enzyme Reg.* 7:149-167 (1969) and Holland et al., "Isolation and Identification of Yeast Messenger Ribonucleic Acids Coding for Enolase, Glyceraldehyde-3-Phosphate Dehydrogenase, and Phosphoglycerate Kinase," *Biochem.* 17:4900-4907 (1978), which are hereby incorporated by referenced in their entirety), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In the construction of suitable expression vectors, the termination sequences associated with these genes are also ligated into the expression vector 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination. Other promoters that have the additional advantage of transcription controlled by growth conditions are the promoter region for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Any plasmid vector containing yeast-compatible promoter, origin of replication and termination sequences is suitable.

[0048] Another representative eukaryotic expression system involves the recombinant baculoviruses, *Autographa californica* nuclear polyhedrosis virus (AcNPV; Summers and Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures* Texas Agriculture Experiment Station. Bulletin No. 1555 (1987) and Luckow et al., "Trends in the Development of Baculovirus Expression Vectors," *Biotechnol.* 6:47-55 (1987), which are hereby incorporated by referenced in their entirety) and *Spodoptera frugiperda*. Baculoviruses do not infect humans and can therefore be safely handled in large quantities.

[0049] A baculovirus expression vector is prepared as previously described using standard molecular biology techniques. The vector may comprise the polyhedron gene promoter region of a baculovirus, the baculovirus flanking sequences necessary for proper crossover during recombination (the flanking sequences comprise about 200-300 base pairs adjacent to the promoter sequence) and a bacterial origin of replication which permits the construct to replicate in bacteria. In particular examples, the vector is constructed so that a heme-binding protein and ferrochelatase nucleic acid sequences are operably linked to the polyhedron gene promoter (collectively, the "expression construct") and the expression construct is flanked by the above-described baculovirus flanking sequences. Appropriate transfer vectors compatible with insect host cells are known in the art and include, without limitation, pVL1392, pVL1393, pAcGP67 and pAcSecG2T, which incorporate asecretory signal fused to the desired protein, and pAcGHLT and pAcHLT, which contain GST and 6×His tags (BD Biosciences, Franklin Lakes, N.J.).

[0050] Insect host cells (e.g., *Spodoptera frugiperda* cells) are infected with a recombinant baculovirus and cultured under conditions allowing expression of the baculovirus-encoded heme-binding proteins and ferrochelatase protein or polypeptide. When using insect cells, suitable baculovirus promoters include late promoters, such as 39K protein promoter or basic protein promoter, and very late promoters, such as the p10 and polyhedron promoters. In some cases it may be desirable to use transfer vectors containing multiple baculoviral promoters. The expressed heme-binding protein may be extracted from the insect cells using methods known in the art.

[0051] Mammalian host cells may also be used for heterologous expression of a heme-binding protein and ferrochelatase protein or polypeptide. Examples of suitable mammalian cell lines include, without limitation, monkey kidney CVI line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line 293S (Graham et al., "Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5," *J. Gen. Virol.* 36:59-74 (1977), which is hereby incorporated by reference in its entirety); baby hamster kidney cells (BHK, ATCC CCL-10); Chinese hamster ovary cells (Urlab et al., "Isolation of Chinese Hamster Cell Mutants Deficient in Dihydrofolate Reductase Activity." *Proc. Natl. Acad. Sci. U.S.A.* 77:4216-4220 (1980), which is hereby incorporated by reference in its entirety); mouse sertoli cells (TM4; Mather J. P., "Establishment and Characterization of Two Distinct Mouse Testicular Epithelial Cell Lines," *Biol. Reprod.* 23:243-252 (1980), which is hereby incorporated by reference in its entirety); monkey kidney cells (CVI-76, ATCC CCL-70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL-2); canine kidney cells (MDCK, ATCC CCL-34); buffalo rat liver cells (BRL 3A, ATCC CRL-1442); human lung cells (W138, ATCC CCL-75); human liver cells (Hep G2, HB 8065); mouse mammary tumor cells (MMT 060562, ATCC CCL-51); rat hepatoma cells (HTC, MI.54; Baumann ct al., "Dexamethasone Regulates the Program of Secretory Glycoprotein Synthesis in Hepatoma Tissue Culture Cells,"*J. Cell Biol.* 85:1-8 (1980), which is hereby incorporated by reference in its entirety); and TRI cells (Mather et al., "Culture of Testicular Cells in Hormone-Supplemented Serum-Free Medium," *Annals N.Y. Acad. Sci.* 383:44-68 (1982), which is hereby incorporated by reference in its entirety). Expression vectors for these cells ordinarily include (if necessary) DNA sequences for an origin of replication, a promoter located 5' of the nucleic acid sequence to be expressed, a ribosome binding site, an RNA splice site, a polyadenylation site, and/or a transcription terminator site.

[0052] Promoters used in mammalian expression vectors can be of viral origin. Such viral promoters may be derived from polyoma virus, adenovirus 2, and simian virus 40 (SV40). The SV40 virus contains two promoters that are

termed the early and late promoters. These promoters are useful because they are both easily obtained from the virus as one nucleic acid fragment that also contains the viral origin of replication (Fiers et al., "Complete Nucleotide Sequence of SV40 DNA," *Nature* 273:113-120 (1978), which is hereby incorporated by reference in its entirety). Smaller or larger SV40 DNA fragments may also be used, provided they contain the approximately 250-bp sequence extending from the HindIII site toward the BglI site located in the viral origin of replication. Alternatively, promoters that are naturally associated with the foreign gene (homologous promoters) may be used provided that they are compatible with the host cell line selected for transformation.

[0053] An origin of replication may be obtained from an exogenous source, such as SV40 or other virus (e.g., polyoma virus, adenovirus, VSV, BPV) and inserted into the expression vector. Alternatively, the origin of replication may be provided by the host cell chromosomal replication mechanism.

[0054] Cell-free translation systems are known in the art, and can be used to synthesize heme-binding proteins using the methods of the present invention (see e.g., Kurland, "Translational Accuracy In vitro," *Cell* 28:201-202 (1982) and Pavlov et al., "Rate of Translation of Natural mRNAs in an Optimized In vitro System,"*Arch. Biochem. Biophys.* 328: 9-16 (1996); and Cell-Free Translation Systems, Spirin A. S., ed. (2002); *Cell-Free Protein Expression*, Swartz J. A., ed. (2003), which are hereby incorporated by reference in their entirety). The most frequently used cell-free translation systems consist of extracts from rabbit reticulocytes, wheat germ and *E. coli*. All are prepared as crude extracts containing all the macromolecular components (70S or 80S ribosomes, tRNAs, aminoacyl-tRNA synthetases, initiation, elongation and termination factors, etc.) required for translation of exogenous RNA. Each extract is supplemented with amino acids, energy sources (ATP, GTP), energy regenerating systems (creatine phosphate and creatine phosphokinase for eukaryotic systems, and phosphoenol pyruvate and pyruvate kinase for the *E. coli* lysate), and other co-factors (Mg^{2+}, K^{+}, etc.) that facilitate the function of the particular translation machinery.

[0055] Either DNA or RNA, in plasmid or linear form, can be used as the starting material for cell-free protein synthesis. However, DNA starting material is necessarily transcribed to RNA using a "coupled" or "linked" system. A "linked" system generally involves DNA transcription with a bacteriophage polymerase followed by translation in the rabbit reticulocyte lysate or wheat germ lysate. Unlike eukaryotic systems (such as, rabbit reticulocyte or wheat germ) where transcription and translation occur sequentially, transcription and translation occur simultaneously in *E. coli* cell free systems. Thus. *E. coli* translation systems are "coupled" and can be performed in the same tube using either a DNA or RNA template. Methods of using *E. coli* cell-free systems have been described in detail (see e.g., Kigawa et al., "Cell-Free Production and Stable-Isotope Labeling of Milligram Quantities of Proteins," *FEBS Lett.* 442:15-19 (1999); Noren et al., "A General Method for Site-Specific Incorporation of Unnatural Amino Acids into Proteins," *Science* 244:182-188 (1989); Hanes et al., "In vitro Selection and Evolution of Functional Proteins by Using Ribosome Display," *Proc. Natl. Acad. Sci. U.S.A.* 94:4937-4942 (1997); Wilson et al., "The Use of mRNA Display to Select High-Affinity Protein-Binding Peptides," *Proc. Natl. Acad. Sci. U.S.A.* 98:3750-3755 (2001); and Sawasaki et al., "A Cell-Free Protein Synthesis System for High-Throughput Proteomics," *Proc. Natl. Acad. Sci. U.S.A.* 99(23):14652-14657 (2002), which are hereby incorporated by reference in their entirety). In the *E. coli* system, it may be advantageous to place a Shine-Dalgarno ribosome binding site upstream of the initiator codon in a DNA template. In particular examples, an *E. coli* S30 extract system allows expression from DNA vectors containing natural *E. coli* promoter sequences (such as lac or tac).

[0056] Another aspect of the present invention relates to a system for producing functional heme-binding proteins. This system comprises an expression system and one or more expression constructs encoding a recombinant heme-binding protein and a recombinant ferrochelatase.

[0057] Suitable expression systems are described above, including cell expression systems (e.g., bacterial, fungal, archaeal, and mammalian) and cell-free expression systems.

[0058] The expression constructs of the system will depend on the type of expression system. Suitable expression constructs are described supra. The expression constructs can be in linear form or contained in a plasmid or viral vector, and can further contain regulatory elements, such as a promoter sequence, a ribosome binding sequence, and a nucleic acid molecule encoding a termination sequence, to optimize protein expression. The system of the present invention can include one expression construct encoding both the recombinant heme-binding protein and ferrochelatase. Alternatively, the system can include a first expression construct encoding the recombinant heme-binding protein and a second expression construct encoding the recombinant ferrochelatase.

[0059] In accordance with this aspect of the invention, the system for producing functional heme-binding proteins further includes one or more heme precursors. Suitable precursors include δ-amino levulinic acid, succinyl CoA, glycine, glutamate, glutamate-1-semialdehyde, porphobilinogen, hydroxymethylbilane and protoporphyrin as described supra.

[0060] Another aspect of the present invention relates to a purified preparation of recombinant functional heme-binding protein.

[0061] In a preferred embodiment of the present invention, the purified preparation of functional recombinant heme-binding protein is prepared in accordance with the methods described supra. The purified preparation of recombinant heme-binding protein of the present invention has full heme incorporation and does not contain metal-free porphyrin. The purity of the preparation is assessed by the presence or absence of un-metallated heme, which can be measured by fluorescence spectroscopy and resonance Raman spectroscopy. In a preferred embodiment of the invention, the purified preparation of functional, recombinant heme-binding protein of the present invention is not fluorescent when excited at a wavelength of 397 nm and has a resonance Raman spectrum showing no evidence of free-base porphyrin incorporation (see Examples infra).

[0062] Purified preparations of heme-binding proteins of the present invention have a variety of therapeutic, research, and commercial utilities. With regard to therapeutic applications, purified preparations of hemoglobin are desired. Hemoglobin is a heme-binding protein responsible for carrying and delivering oxygen to tissues and organs in animals. Recombinant hemoglobin preparations are used as effective and safe oxygen carriers as an alternative to blood transfusion. A purified preparation of recombinant hemoglobin prepared in accordance with the methods of the present invention

is fully functional due to full heme incorporation. Accordingly, the incorporation of a purified preparation of hemoglobin of the present invention into oxygen carrier and blood substitute technologies would improve the oxygen carrying capacity. Suitable oxygen carrier and blood substitute technologies include, without limitation, those disclosed in U.S. Pat. No. 4,412,989 to Iwashits et al., U.S. Pat. No. 6,022,849 to Olsen et al, U.S. Pat. No. 7,329,641 to Fronticellie et al., and U.S. Patent Publication No. 2006/0088583 to Takeoka et al., which are hereby incorporated by reference in their entirety.

[0063] Cytochrome P450s are a superfamily of enzymes that are critical to human drug metabolism. These proteins have been implicated in many clinical cases of adverse drug reaction and toxicity stemming from mechanism-based enzyme inhibition and drug-drug interactions. High-throughput assays that identify molecules that inhibit or induce CYP450 early in the drug development process are invaluable for guiding the elimination of candidate drugs that have unwanted metabolic properties and facilitating the production of better clinical candidates. Purified preparations of cytochrome P450 proteins of the present invention will benefit the variety of well established preclinical screening assays that are designed to predict drug metabolism and toxicity (see e.g., Trubetskoy et al., "Highly Minaturized Formats for In Vitro Drug Metabolism Assays Using Vivid Fluorescent Substrate and Recombinant Human Cytochrome P450 Enzymes," *J Bimolecular Screening* 56-66 (2005); Donato et al., "Fluorescence-Based Assays for Screening Nine Cytochrome P450 (P450) Activities in Intact Cells Expression Individual Human P450 Enzymes," *Drug Metab. Disposition* 32(7):699-706 (2004); Zhang et al., "Cytochrome P450 Reaction-Phenotyping: An Industrial Perspective," Expert Opin, Drug Metab. Toxicol. 3(5):667-87 (2007); Buters et al., "A Highly Sensitive Tool for the Assay of Cytochrome P450 Enzyme Activity in Rat, Dog, and Man: Direct Fluorescence Monitoring of the Deethylation of 7-ethoxy-4-trifluoromethylcoumarin," *Biochem. Pharm.* 46(20):1577-1584 (1993); and Yim et al., "A Continuous Spectrophotometric Assay for NADPH-cytochrome P450 Reductase Activity Using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide," *J. Biochem. Mol. Biol.* 38(3):366-369 (2005), which are hereby incorporated by reference in their entirety).

[0064] Purified preparations of nitric oxide synthases of the present invention also have therapeutic utility with regard to the treatment of vascular diseases, cancer, microbial infections, tissue injury, and neurological pathologies, and for promoting wound healing (see e.g., U.S. Pat. No. 5,658,565 to Billiar et al., U.S. Patent Publication No. 20070071725 to Paterson et al., and U.S. Patent Publication No. 20100087370 to Jain et al., which are hereby incorporated by reference in their entirety).

[0065] Purified preparations of cyclooxygenases (e.g. COX-1, COX-2, COX-3) of the present invention also have therapeutic/commercial utility. These enzymes mediate the formation of prostanoids, including prostaglandins, prostacyclin, and thromboxane. Pharmacological inhibition of these enzymes provides relief from the symptoms of inflammation and pain. Accordingly, purified preparations of cyclooxygenases made in accordance with the present invention can be used to screen and identify, using methods described infra, more potent inhibitors than the currently available non-steroidal anti-inflammatory drugs.

[0066] In addition to therapeutic utility, purified preparations of heme-binding proteins also have biotechnological utilities. For example, lignin peroxidase is a heme-binding protein that degrades plant lignin, a heterogenous aromatic polymer that encases the cellulose fibers of lignocellulose (see Weng et al., "Emerging Strategies in Lignin Engineering and Degradation for Cellulosic Biofuel Production," *Curr. Opin. Biotech.* 19:166-172 (2008), which is hereby incorporated by reference in its entirety). The lignocellulose content of plant biomass is a primary source of renewable carbon that can be used to produce bio-ethanol and chemical feedstocks for commercial use. A major obstacle to exploiting this renewable carbon source is the presence and degradation of lignin. Therefore, lignin degradation via lignin peroxidase, offers an attractive strategy for optimizing biofuel production (see e.g., U.S Patent Application Publication Nos. 2010/0291653 to Ness et al., 2010/0017916 to Pappan et al., 2005/0233423 to Berka et al., which are hereby incorporated by reference in their entirety). Accordingly, the present invention contemplates the use of a purified preparation of lignin peroxidase that does not contain metal-free porphyrin, for biofuel production.

[0067] Another aspect of the present invention relates to a method of identifying an agent that modulates activity of a heme-binding protein. This method involves providing a candidate agent and providing a recombinant functional heme-binding protein. This method further involves contacting the candidate agent with the recombinant functional heme-binding protein under conditions at which the functional heme-binding protein is active and comparing the activity of the functional heme-binding protein as a result of said contacting to the activity of the heme-binding protein alone, both under said conditions at which the heme-binding protein is active. A candidate agent that modulates the activity of a heme-binding protein is identified based on said comparing.

[0068] A related aspect of the present invention relates to a method of evaluating the metabolism of an agent by a heme-binding protein. This method involves providing a candidate agent and providing a recombinant functional heme-binding protein. This method further involves contacting the candidate agent with the recombinant functional heme-binding protein under conditions at which the functional heme-binding protein is active and comparing the activity of the functional heme-binding protein as a result of said contacting to the activity of the heme-binding protein alone, both under said conditions at which the heme-binding protein is active. The metabolism of the candidate agent by a heme-binding protein is evaluated based on said comparing.

[0069] In one embodiment of this aspect of the present invention, the above methods further involve providing a heme-binding protein substrate and/or one or more heme-binding protein co-factors, where the heme-binding protein substrate and/or heme-binding co-factor is present when the heme-binding protein is contacted with the candidate agent. The activity of the heme-binding protein in the presence of the one or more heme-binding cofactors with the heme-binding protein substrate is evaluated. In other words, the rate of metabolism or conversion of the heme-binding protein substrate by the heme-binding protein is a measure of heme-binding protein activity.

[0070] In accordance with this aspect of the present invention a decrease in the activity of the functional heme-binding protein in the presence of the candidate agent compared to in the absence of the candidate agent identifies an agent that

modulates the heme-binding protein activity. In this regard, the candidate agent is an inhibitor of heme-binding protein activity. Activators of heme-binding protein activity can also be identified using this assay. An increase in heme-binding protein activity would identify candidate activators of heme-binding protein activity.

[0071] In accordance with aspects of the present invention directed to evaluating the metabolism of an agent by a heme-binding protein, an increase in the activity of a functional heme-binding protein in the presence of the candidate agent compared to in the absence of the candidate agent may identify an agent that is a substrate for heme-binding protein metabolism. Alternatively, a decrease in heme-binding protein activity may also indicate that the candidate agent is a substrate for the heme-binding protein activity (e.g., a substrate for cytochrome P450 metabolism). In this case, the observed decrease in heme-binding protein activity would result from competition for heme-protein binding between the heme-binding protein substrate and the candidate agent. In either case, metabolism of the candidate agent by the heme-binding protein can be further evaluated by analyzing the metabolic profile of the candidate agent using methods known in the art (e.g., high-performance liquid chromatography).

[0072] In accordance with these aspects of the present invention, the recombinant functional heme-binding protein does not contain metal-free porphyrin. Any of the heme-binding proteins described supra can be utilized in this aspect of the invention. In one embodiment of the present invention, the heme-binding protein is a cytochrome P450 protein. As noted above, the cytochrome P450 family of enzymes are the major catalysts for the oxidative metabolism of a vast array of hydrophobic chemicals. These enzymes are involved in the metabolism or biotransformation of endogenous as well as exogenous hydrophobic compounds. Since cytochrome P450-mediated metabolism influences drug clearance, toxicity, activation, and in some cases, adverse interaction with other drugs, accurately identifying agents that modulate cytochrome P450 activity or serve as substrates for metabolism is particularly important for early toxicological screening of candidate drugs. A number of cytochrome P450 screening assays are known in the art (see e.g., Donato et al., "Fluorescence-Based Assays for Screening Nine Cytochrome P450 (P450) Activities in Intact Cells Expression Individual Human P450 Enzymes," *Drug Metab. Disposition* 32(7):699-706 (2004); Zhang et al., "Cytochrome P450 Reaction-Phenotyping: An Industrial Perspective," Expert Opin. Drug Metab. Toxicol, 3(5):667-87 (2007); Buters et al., "A Highly Sensitive Tool for the Assay of Cytochrome P450 Enzyme Activity in Rat, Dog, and Man: Direct Fluorescence Monitoring of the Deethylation of 7-ethoxy-4-trifluoromethylcoumarin," *Biochem, Pharm.* 46(20):1577-1584 (1993); and Yim et al., "A Continuous Spectrophotometric Assay for NADPH-cytochrome P450 Reductase Activity Using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide," *J. Biochem. Mol. Biol.* 38(3066-369 (2005), which are hereby incorporated by reference in their entirety) and are commercially available, especially in high-throughput formats (see e.g. Promega. Invitrogen, and Agilent Technologies), all of which can be utilized in these aspects of the present invention, Incorporation of purified preparations of recombinant cytochrome P450 proteins of the present invention will enhance the accuracy of these and other P450 enzyme assays known and used in academic and pharmaceutical research arenas.

EXAMPLES

[0073] The following examples illustrate various methods for compositions in the treatment method of the invention. The examples are intended to illustrate, but in no way limit, the scope of the invention.

Materials and Methods for Examples 1-3:

[0074] Co-expression of Ferrochelatase with gsNOS. Ferrochelatase (FC) and gsNOS were expressed from the same pACYCduet vector (Novagen). To clone FC, genomic DNA was extracted from *E. coli* BL21(DE3) cells with the genomic DNA extraction kit from Epicenter. The FC gene was then PCR-amplified (Phusion polymerase, New England Biolabs) from the genomic DNA with primers that generated Nde1 and Xho1 sites at the 5' and 3' ends of the gene, respectively. A stop codon was introduced into the 3' primer before the Xho1 site to prevent C-terminal attachment of the vector-supplied S-tag. The amplified FC gene was then cloned into the Nde1 and Xho1 sites in Multiple Cloning Site-2 of the pACYCdtiet vector. The gene for gsNOS was derived from a previous pET28a-gsNOS plasmid (Sudhamsu et al., "Structure and Reactivity of a Thermostable Prokaryotic Nitric-Oxide Synthase that Forms a Long-Lived Oxy-Heme Complex," *J. Biol. Chem.* 281:9623-9632 (2006), which is hereby incorporated by reference in its entirety) by digesting the vector with Nco1 and Xho1 so as to include the His-tag and the thrombin cleavage site along with the coding sequence for gsNOS in the excised fragment. The Nco1-Xho1 fragment was then cloned into the pACYCduet-FC plasmid between the Nco1 and Sal1 sites. Sal1 and Xho1 produce compatible cohesive ends and thereby allow the His-tag, thrombin cleavage site and gsNOS fragment to be cloned between the Nco1 and Sal1 sites of Multiple cloning site-1 of the pACYCduct-FC plasmid. The resulting pACYCduet plasmid allows over-expression of gsNOS with a cleavable His-tag and FC with no tag, GsNOS was expressed and purified as reported before (Sudhamsu et al., "Structure and Reactivity of a Thermostable Prokaryotic Nitric-Oxide Synthase that Forms a Long-Lived Oxy-Heme Complex," *J. Biol. Chem.* 281:9623-9632 (2006), which is hereby incorporated by reference in its entirety). Co-expression of gsNOS and FC was also performed similarly to expression of gsNOS alone, although, a lesser amount of δ-ALA was added at the time of induction (10 mg/L versus 25 mg/L for gsNOS), and the growth media was supplemented with 100 μM $FeCl_3$. The antibiotics, chloramphenicol (34 μg/L) and kanamycin (50 μg/L), were added to the growth media of pACYCduet-gsNOS-FC and pET28a-gsNOS plasmids, respectively.

[0075] Co-expression of Ferrochelatase with BP450 and HBPAS. For co-expression of BP450 and HBPAS the same procedure was used. BP450 (NCBI: CBG70284) was cloned into pET151/D-TOPO (Invitrogen), a directional cloning vector with an N-terminal 6×His-Tag followed by a TEV cleavage site and an ampicillin selectable marker. HBPAS (NCBI: NP_248866) was cloned into pET28a (Novagen) using NdeI and HindIII restriction sites, which included an N-terminal 6×His-Tag followed by a thrombin cleavage site and a kanamycin selectable maker. Competent *E. coli* BL21 (DE3) cells containing FC/pACYCduet were transformed with either BP450/pET151/D-TOPO or HBPAS/pET28.

Cells were grown at 37° C. in Luria broth containing 20 ug/mL Cm and 100 ug/L Amp (BP450) or 50 ug/L Kan (HBPAS) to an OD=0.6-0.8, Prior to induction with IPTG, the temperature was reduced to 17° C. and 25 mg/L d-ALA was added to the growth media. Cells were harvested 18-20 hrs after induction. An identical procedure with cells lacking the FC plasmid was used to express BP450 and HBPAS without FC. Both proteins were purified using Ni-NTA (Qiagen) chromatography techniques following the manufacturer's protocol. Furthermore, the proteins were purified to >95% purity using size exclusion chromatography after the removal of 6xHis.

[0076] Spectroscopy. Resonance Raman and UV-Visible spectra were recorded as described previously (Kabir et al., "Substrate-Ligand Interactions in *Geobacillus Stearothermophilus* Nitric Oxide Synthase," *Biochem.* 47:12389-12397 (2008), which is hereby incorporated by reference in its entirety).

[0077] Materials. Sodium chloride was obtained from Mallinkrodt, Ferric Chloride, IPTG and TRIS were from Fisher Scientific, Kanamycin, and Chloramphenicol from USBiological. δ-ALA was obtained from Sigma-Aldrich.

Example 1

UV-Vis Spectroscopy and SDS-PAGE Analysis of gsNOS

[0078] GsNOS (*Geobacillus stearothermophihis* Nitric Oxide Synthase) is a thermophilic enzyme that forms a highly stable heme-oxygen complex (Sudhamsu et al., "Structure and Reactivity of a Thermostable Prokaryotic Nitric-Oxide Synthase that Forms a Long-Lived Oxy-Heme Complex,"*J. Biol. Chem.* 281:9623-9632 (2006), which is hereby incorporated by reference in its entirety) that has helped in identification of catalytic intermediates responsible for L-arginine oxidation to nitric oxide (Davydov et al., "EPR And ENDOR Characterization of the Reactive Intermediates in the Generation of NO by Cryoreduced Oxy-Nitric Oxide Synthase from G. Stearotherniophilus,"*J. Am. Chem. Soc.* 131:14493-14507 (2009), which is hereby incorporated by reference in its entirety). In the over-expression of heme proteins the heme precursor δ-ALA is routinely added to the growth media when protein production is induced. Such δ-ALA supplementation results in complete heme incorporation for two other bacterial NOS proteins: *B. subtilis* NOS (Pant et al., "Structure of a Nitric Oxide Synthase Heme Protein from *Bacillus Subtilis," Biochem.* 41:11071-11079 (2002), which is hereby incorporated by reference in its entirety) and *D. radiodurans* NOS (Buddha et al., "Regioselective Nitration of Tryptophan by a Complex Between Bacterial Nitric-Oxide Synthase and Tryptophanyl-Trna Synthetase," *J. Biol. Chem.* 279:49567-49570 (2004), which is hereby incorporated by reference in its entirety). However, in what follows, it is shown that gsNOS over-expressed and purified from *E. coli* consists of two species: native heme-containing gsNOS, and gsNOS with protoporphyrin IX (free-base porphyrin) bound instead of heme. It was hypothesized that co-expression of ferrochelatase, the enzyme that metallates porphyrin would ameliorate this problem.

[0079] A UV-Vis spectroscopic analysis of gsNOS over-expressed in *E. coli* shows that the amount of heme incorporated with the protein changes from batch to batch, with the ratio of Soret peak height (403 nm) to protein peak height (280 nm) (Abs_{403}/Abs_{280}) varying between 0.25-0.40. Co-expression of FC with gsNOS increased Abs_{403}/Abs_{280} to 0.6 (FIG. **1**) in several (>3) different protein expression trials. Thus, gsNOS co-expressed with FC increases heme content of the protein in a consistent fashion.

[0080] Purified gsNOS, when over-expressed in *E. coli* with 25 mg/L δ-ALA added at the time of induction, results in two bands of ~42 kDa on SDS-PAGE (FIG. **1**, Inset, lane A). Both bands shift on His-tag cleavage (FIG. **1**, Inset, lane B), which indicates that both species represent recombinant gsNOS, with an intact N-terminus, but different gel mobilities. MALDI mass spectrometry of the sample shows only one sharp peak at a mass consistent with that of full-length gsNOS, which rules out proteolysis as the factor distinguishing the proteins represented in the two bands. Thus, the apparent difference in molecular weight on the gel of the two species (~3 kDa) may rather stem from a net charge difference. GsNOS, when co-expressed with FC from *E. coli* BL21 (DE3) cells, results in only a single species on SDS-PAGE (which corresponds to the lower band of the two observed previously; FIG. **1** Inset, lane C). On His-tag cleavage, this band shifts as a single species (FIG. **1**, Inset, lane D). Protoporphyrin IX is the penultimate product in the heme biosynthesis pathway. FC catalyzes the last step in heme biosynthesis, i.e., insertion of an iron atom into protoporphyrin IX. Thus, the presence of two bands in the absence of FC co-expression suggests that protoporphyrin IX, rather than heme, has been incorporated into a substantial fraction of the sample.

Example 2

Resonance Raman and Fluorescence Analysis of gsNOS

[0081] Confirmation of porphyrin incorporation into gsNOS came from resonance Raman studies of gsNOS in the presence of substrate L-arginine. A sample of gsNOS (expressed without FC) in the presence of substrate L-arginine, shows vibrational frequencies at 662 cm^{-1}, 738 cm^{-1}, 783 cm^{-1}, 1360 cm^{-1} and 1543 cm^{-1}, apart from the typical vibrational frequencies that have been previously observed (Santolini et al., "Resonance Raman Study of *Bacillus Subtilis* NO Synthase-Like Protein: Similarities and Differences with Mammalian NO Synthases," *Biochem.* 45:1480-1489 (2006) and Rousseau et al., "Ligand-Protein Interactions in Nitric Oxide Synthase," *J. Inorganic Biochem.* 99:306-323 (2005), which are hereby incorporated by reference in their entirety) for other NOSs (FIG. **2**B). These additional bands disappear after exposure to laser (FIG. **2**C) and the subsequent difference spectrum (before and after laser exposure) highlights the original, additional resonances (FIG. **2**D). The frequencies of these vibrations indicates the presence of free-base porphyrin in the protein sample (Blackwood et al., "Alternative Modes of Substrate Distortion in Enzyme and Antibody Catalyzed Ferrochelation Reactions," *Biochem.* 37:779-782 (1998) and Lu et al., "Binding of Protoporphyrin IX and Metal Derivatives to the Active Site of Wild-Type Mouse Ferrochelatase at Low Porphyrin-to-Protein Ratios," *Biochem.* 41:8253-8262 (2002), which are hereby incorporated by reference in their entirety). Furthermore, the enhanced photosensitivity of porphyrin compared to heme explains why the bands disappear after exposure (Blackwood et al., "Alternative Modes of Substrate Distortion in Enzyme and Antibody Catalyzed Ferrochelation Reactions," *Biochem.* 37:779-782 (1998) and Lu et al., "Binding of Protoporphyrin IX and Metal Derivatives to

the Active Site of Wild-Type Mouse Ferrochelatase at Low Porphyrin-to-Protein Ratios," *Biochem.* 41:8253-8262 (2002), which are hereby incorporated by reference in their entirety). The presence of free base porphyrin is corroborated by the fluorescence spectrum of gsNOS (FIG. **2**E), which when measured with excitation at 397 nm shows definitive characteristics of un-metallated heme (Lozovaya et al., "Protoporphyrin-IX as a Possible Ancient Photosensitizer—Spectral and Photochemical Studies," *Origins Of Life And Evolution Of The Biosphere* 20:321-330 (1990), which is hereby incorporate by reference in its entirety). Iron-bound heme is not fluorescent when excited at this wavelength. Incorporation of free-base porphyrin suggests that the last step of heme biosynthesis, i.e., Fe metallation to heme, which is performed by FC, cannot keep pace with protein folding and porphyrin incorporation. Co-expression of FC from *E. coli* with gsNOS and addition of 10 mg/L of δ-ALA of (60 μM, ~$0.50 per liter) generates a sample that is non-fluorescent and whose resonance Raman spectrum shows no evidence of porphyrin (FIG. **2**A). Thus, under these conditions, the protein is fully heme incorporated.

[0082] Whether foregoing the addition of δ-ALA was also tested, while co-expressing FC would result in complete heme incorporation of gsNOS. The purified protein, which was checked for heme content by both SDS-PAGE and by UV-Vis spectroscopy had a higher degree of home incorporation than under conditions of only adding δ-ALA (heme: protein ratio ~0.5); however, heme incorporation was not complete, Addition of a small amount of δ-ALA, (10 mg/L) is sufficient to make up for the slow rate of δ-ALA biosynthesis and produce fully incorporated protein in the presence of FC.

Example 3

FC-Assisted Heme Incorporation for a Bacterial P450 (BP450) and a Heme-Binding PAS Protein (HBPAS)

[0083] In addition to gsNOS, FC also increases heme content to saturating levels in two other unrelated proteins: BP450, a Cys-ligated heme protein and HBPAS: a His-ligated heme protein. Both of these proteins, when over-expressed in *E. coli*, are produced with partial heme incorporation, UV-Vis spectra of purified BP450 and BP450 co-expressed with FC are strikingly different, with the increased intensity of the Soret peak indicative of greater heme content in the material produced along with FC (FIG. **3**), When analyzed by SDS-PAGE, BP450 again also shows two bands (FIG. **3**, Inset, lane A), which both shift on His-tag cleavage (FIG. **3**, Inset, lane B). Like gsNOS, on co-expression with FC (FIG. **3**, Inset, lane C) BP450 produces only one band, which shifts on His-tag cleavage (FIG. **3**, Inset, lane D). In contrast, HBPAS always results in a single band on a SDS-PAGE gel, but shows absorption for four Q-bands in the UV-Vis spectrum when produced without FC (FIG. **4**, thin line). Pure heme proteins show only two such bands. The extra band(s) are representative of protein bound protoporphyrin IX (Lozovaya et al., "Protoporphyrin-IX as a Possible Ancient Photosensitizer—Spectral and Photochemical Studies," *Origins Of Life And Evolution Of The Biosphere* 20:321-330 (1990), which is hereby incorporated by reference in its entirety). Co-expression of FC results in an increase in the $Abs_{(Soret)}/Abs_{(280)}$ and the extra Q-bands disappear (FIG. **4**, thick line). The fluorescence spectrum of HBPAS without co-expression of FC is similar to that of gsNOS expressed without FC, but this fluorescence, attributable to protoporphyrin IX, also disappears on co-expression with FC.

Discussion of Examples 1-3

[0084] The production of δ-ALA is a rate-limiting step for heme biosynthesis (Ades, I. Z., "Heme Production in Animal-Tissues—The Regulation of Biogenesis of Delta-Aminolevulinate Synthase," *Internat. Biochem.* 22:565-578 (1990); Woodard et al., "Regulation of Heine-Biosynthesis in *Escherichia-Coli," Archives Biochem. Biophys.* 316:110-115 (1995); Gibson et al., "Is Delta-Aminolevulinic Acid Dehydratase Rate Limiting in Heme Biosynthesis Following Exposure of Cells to Delta-Aminolevulinic Acid?" *Photochem. Photobiol.* 73:312-317 (2001); and Heinemann et al., "The Biochemistry of Heme Biosynthesis," *Archives Biochem. Biophys.* 474:238-251 (2008), which are hereby incorporated by reference in their entirety) and δ-ALA synthesis is itself slowed by heme feedback inhibition. Thus, as has been well recognized (Ades, I. Z., "Heme Production in Animal-Tissues—The Regulation of Biogenesis of Delta-Aminolevulinate Synthase,"*Internat. J. Biochem.* 22:565-578 (1990); Woodard et al., "Regulation of Heme-Biosynthesis in *Escherichia-Coli," Archives Biochem. Biophys.* 316:110-115 (1995); Gibson et al., "Is Delta-Aminolevulinic Acid Dehydratase Rate Limiting in Heme Biosynthesis Following Exposure of Cells to Delta-Aminolevulinic Acid?" *Photochem. Photobtol.* 73:312-317 (2001); and Heinemann et al., "The Biochemistry of Heme Biosynthesis,"*Archives Biochem. Biophys.* 474:238-251 (2008), which are hereby incorporated by reference in their entirety), feeding with δ-ALA greatly aids recombinant heme protein production in *E. coli*. However, as demonstrated herein, under conditions of augmentation with δ-ALA, ferrous iron insertion into protoporphyrin IX becomes rate-limiting. Co-expression with ferrochelatase along with the addition of a small amount of δ-ALA, is sufficient to produce fully incorporated heme protein. This method is applicable for both Cys-ligated and His-ligated heme proteins. In the case of the two Cys-ligated proteins, porphyrin substitution could be observed on an SDS-PAGE gel as two closely spaced bands and also by fluorescence spectroscopy. In the one His-ligated heme protein example, UV-Visible and fluorescence spectra were effective indicators of insufficient porphyrin metallation, but only one band was observed by SDS-PAGE even with less than full porphyrin content. This is probably because the heme or porphyrin does not remain associated with the PAS protein during electrophoresis, unlike the other two cases.

[0085] In conclusion, the simple and inexpensive method of co-expressing ferrochelatase is effective at producing fully incorporated heme proteins in *E. coli*.

Example 4

FC-Assisted Heme Incorporation for Full-Length NOS

[0086] Co-expression of FC and full-length NOS was carried out as described supra. Under normal conditions of expression the Soret band indicative of heme incorporation is barely visible above the flavin absorption of the reductase component (gray trace), however, upon co-expression with ferrochelatase a strong Soret absorption peak (at ~416 nm), indicative of heme incorporation becomes apparent (black

trace). Total yields of full-length NOS also increase in the presence of ferrochelatase as indicated by the overall greater cofactor absorption profile.

[0087] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

What is claimed is:

1. A method of producing a functional recombinant heme-binding protein comprising:

co-expressing a recombinant heme-binding protein and recombinant ferrochelatase protein or polypeptide thereof under conditions effective for complete heme incorporation into the recombinantly produced heme-binding protein, thereby producing a functional heme-binding protein.

2. The method of claim **1**, wherein said co-expressing is carried out in the presence of one or more heme precursors.

3. The method of claim **2**, wherein the heme precursor is selected from the group consisting of δ-amino levulinic acid, succinyl CoA, glycine, glutamate, glutamate-1-semialdehyde, porphobilinogen, hydroxymethylbilane, and protoporphyrin.

4. The method of claim **1**, wherein said co-expressing is carried out in a cell-free expression system.

5. The method of claim **1**, wherein said co-expressing is carried out in a host cell.

6. The method of claim **5**, wherein the host cell is selected from the group consisting of a bacterial cell, fungal cell, insect cell, plant cell, archaeal cell, and mammalian cell.

7. The method of claim **6**, wherein the host cell is a bacterial cell selected from the group consisting of *Escherichia coli, Bacillus subtilis, B. mojavensis, B. atrophaeus, B. licheniformis, Salmonella typhimurium, Serratia marcesans, Pseudomonas aerurginosa*, and *Rhodobacter spheroids.*

8. The method of claim **1** further comprising:

providing one or more expression vectors encoding the recombinant heme-binding protein and the recombinant ferrochelatase.

9. The method of claim **8**, wherein the one or more expression vectors comprise one expression vector encoding both the recombinant heme-binding protein and the recombinant ferrochelatase.

10. The method of claim **8**, wherein the one or more expression vectors comprise a first expression vector encoding the recombinant heme-binding protein and a second expression vector encoding ferrochelatase.

11. The method of claim **8**, wherein the one or more expression vectors is selected from the group consisting of a bacterial expression vector, fungal expression vector, baculoviral expression vector, plant expression vector, archaeal expression vector, and mammalian expression vector.

12. The method of claim **1**, wherein the heme-binding protein is a mammalian heme-binding protein.

13. The method of claim **1**, wherein the heme-binding protein is a bacterial heme-binding protein.

14. The method of claim **1**, wherein the heme-binding protein is a member of a class of heme-binding proteins selected from the group consisting of globins, cytochromes, bacterioferririns, hydroxylamine oxidoreductases, nitrophorins, peroxidases, cyclooxygenases, catalases, cytochromes P-450s, chloroperoxidases, PAS-domain heme sensors, H-NOX heme sensors, and nitric oxide synthases.

15. The method of claim **1**, wherein the heme-binding protein does not contain metal-free porphyrin.

16. A system for producing functional heme-binding proteins comprising:

an expression system and

one or more expression constructs encoding a recombinant heme-binding protein and a recombinant ferrochelatase.

17. The system of claim **16** further comprising:

one or more heme precursors.

18. The system of claim **17**, wherein the one or more heme precursors is selected from the group consisting of δ-amino levulinic acid, succinyl CoA, glycine, glutamate, glutamate-1-semialdehyde, porphobilinogen, hydroxymethylbilane, and protoporphyrin.

19. The system of claim **16**, wherein the expression system is a cell-free expression system.

20. The system of claim **16**, wherein the expression system comprises a host cell.

21. The system of claim **20**, wherein the host cell is selected from the group consisting of a bacterial cell, fungal cell, insect cell, plant cell, archaeal cell, and mammalian cell.

22. The system of claim **16**, wherein the one or more expression constructs further comprise a promoter sequence, a nucleic acid encoding a ribosome binding sequence, and a nucleic acid encoding a termination sequence.

23. The system of claim **16**, wherein the one or more expression constructs is selected from the group consisting of a linear DNA construct, a plasmid vector, and a viral vector.

24. The method of claim **16**, wherein the one or more expression vectors comprise one expression construct encoding both the recombinant heme-binding protein and the recombinant ferrochelatase.

25. The method of claim **16**, wherein the one or more expression vectors comprise a first expression construct encoding the recombinant heme-binding protein and a second expression construct encoding ferrochelatase.

26. The method of claim **16**, wherein the one or more expression constructs is selected from the group consisting of a bacterial expression vector, fungal expression vector, baculoviral expression vector, plant expression vector, archaeal expression vector, and mammalian expression vector.

27. The method of claim **16**, wherein the heme-binding protein is a mammalian heme-binding protein.

28. The method of claim **16**, wherein the heme-binding protein is a bacterial heme-binding protein.

29. The method of claim **16**, wherein the heme-binding protein is a member of a class of heme-binding proteins selected from the group consisting of cytochromes, bacterioferririns, hydroxylamine oxidoreductases, nitrophorins, peroxidases, cyclooxygenases, catalases, cytochromes P-450s, chloroperoxidases, globins, PAS-domain heme sensors, H-NOX heme sensors, and nitric oxide synthases.

30. The method of claim **16**, wherein the heme-binding protein does not contain metal-free porphyrin.

31. A purified preparation of recombinant functional heme-binding protein.

32. The purified preparation of claim **31**, wherein said recombinant functional heme-binding protein does not contain metal-free porphyrin.

33. The purified preparation of claim **31**, wherein the recombinant functional heme-binding protein is a mammalian heme-binding protein.

34. The purified preparation of claim **31**, wherein the recombinant functional heme-binding protein is a bacterial heme-binding protein.

35. The purified preparation of claim **31**, wherein the recombinant functional heme-binding protein is a member of a class of heme-binding proteins selected from the group consisting of cytochromes, bactcrioferririns, hydroxylamine oxidoreductases, nitrophorins, peroxidases, cyclooxygenases, catalases, cytochromes P-450s, chloroperoxidases, globins, PAS-domain heme sensors, H-NOX heme sensors, and nitric oxide synthases.

36. A method of identifying an agent that modulates activity of a heme-binding protein comprising:

providing a candidate agent;

providing a recombinant functional heme-binding protein;

contacting the candidate agent with the recombinant functional heme-binding protein under conditions at which the functional heme-binding protein is active;

comparing the activity of the functional heme-binding protein as a result of said contacting to the activity of the heme-binding protein alone, both under said conditions at which the heme-binding protein is active; and

identifying a candidate agent that modulates the activity of a heme-binding protein based on said comparing.

37. The method of claim **36**, wherein the recombinant functional heme-binding protein does not contain metal-free porphyrin.

38. The method of claim **36**, wherein the recombinant functional heme-binding protein is a cytochrome P450 protein.

39. The method of claim **36** further comprising:

providing a heme-binding protein substrate, where the heme-binding protein substrate is subjected to said contacting and the activity of the heme-binding protein in the presence of the heme-binding protein substrate is evaluated during said comparing.

40. The method of claim **36** further comprising:

providing one or more heme-binding protein co-factors, where the heme-binding protein co-factor is subjected to said contacting and the activity of the heme-binding protein in the presence of the heme-binding co-factor is evaluated during said comparing.

41. The method of claim **36**, wherein a decrease in the activity of the functional recombinant heme-binding protein in the presence of the candidate agent compared to in the absence of the candidate agent identifies an agent that inhibits the heme-binding protein activity.

42. A method of evaluating the metabolism of an agent by a heme-binding protein comprising:

providing a candidate agent;

providing a recombinant functional heme-binding protein;

contacting the candidate agent with the recombinant functional heme-binding protein under conditions at which the functional heme-binding protein is active;

comparing the activity of the functional heme-binding protein as a result of said contacting to the activity of the heme-binding protein alone, both under said conditions at which the heme-binding protein is active; and

evaluating the metabolism of the candidate agent by a heme-binding protein based on said comparing.

43. The method of claim **42**, wherein the recombinant functional heme-binding protein does not contain metal-free porphyrin.

44. The method of claim **42**, wherein the recombinant function heme-binding protein is a cytochrome P450 protein.

45. The method of claim **42** further comprising:

providing one or more heme-binding protein co-factors where the heme-binding protein co-factor is subjected to said contacting and the activity of the heme-binding protein in the presence of the heme-binding co-factor is evaluated during said comparing.

46. The method of claim **42**, wherein an increase in the activity of the functional heme-binding protein in the presence of the candidate agent compared to in the absence of the candidate agent identifies an agent that is a substrate for metabolism by a heme-binding protein.

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