Supporting Information

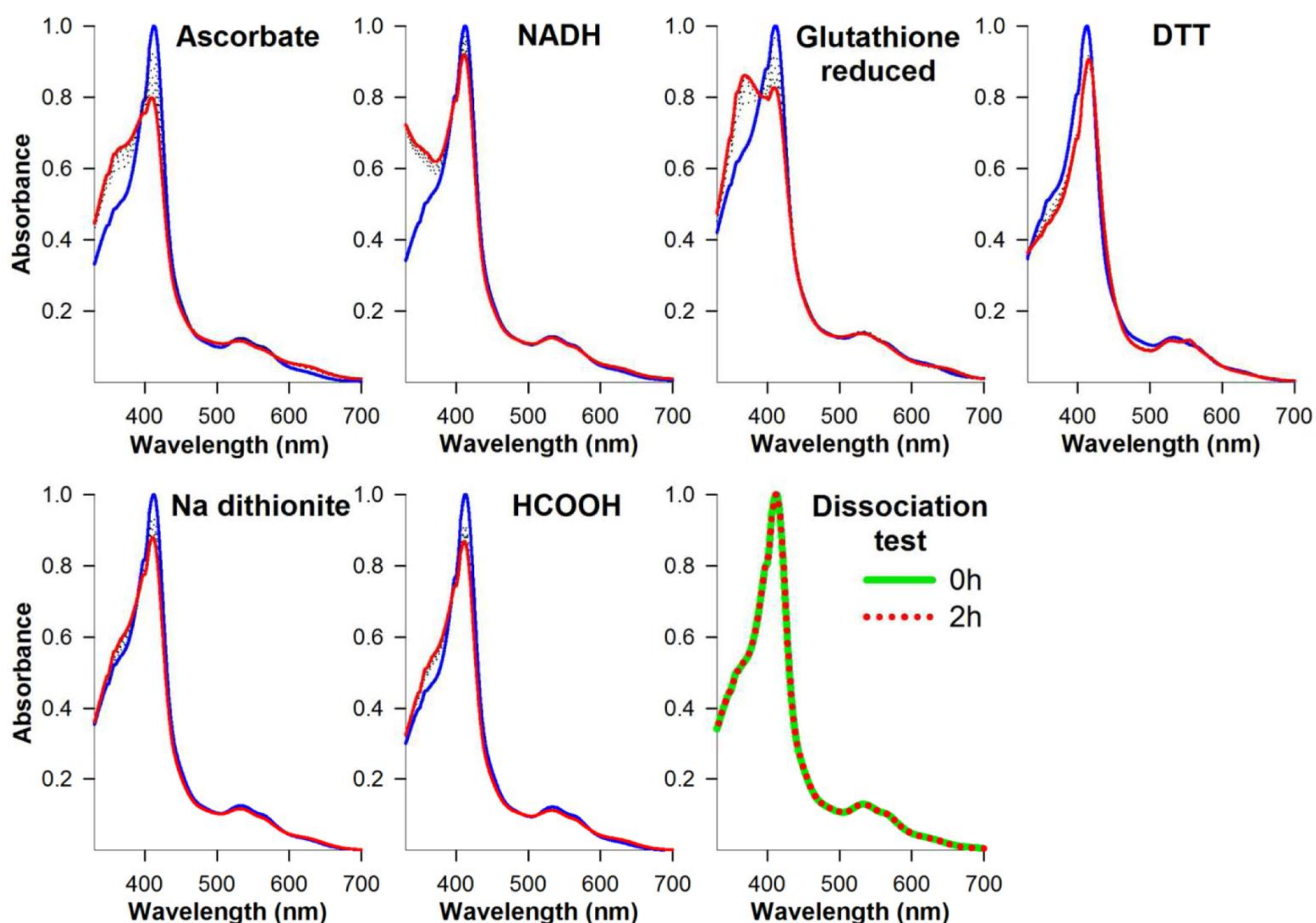

Figure S1. Heme-degrading activity of Isd-LmHde in the presence of various electron donor sources. Absorption spectra of 10 μM hemin-protein complex in the presence of different reductants and catalase at a molar ratio of 0.5:1.0 (catalase:hemoprotein). Spectra were taken immediately before addition of 10 mM each reductant and then every 5 min. The name of each reductant was denoted above each set of spectra. The spectrum before the addition of reductants is shown as a solid blue line (——), spectra of the intermediate time points (every 5 min) after the addition of reductants are shown as dotted lines (……), and the spectrum at 30 min is shown as solid red line (——). The dissociation of the hemin-Isd-LmHde complex is checked over 2h in the absence of reductants. The spectrum of the hemoprotein complex at 0h is shown as a solid green line and the spectrum at 2h is shown as

a dotted red line. These maximum absorption values were normalized to 1.0 for clearer comparison.

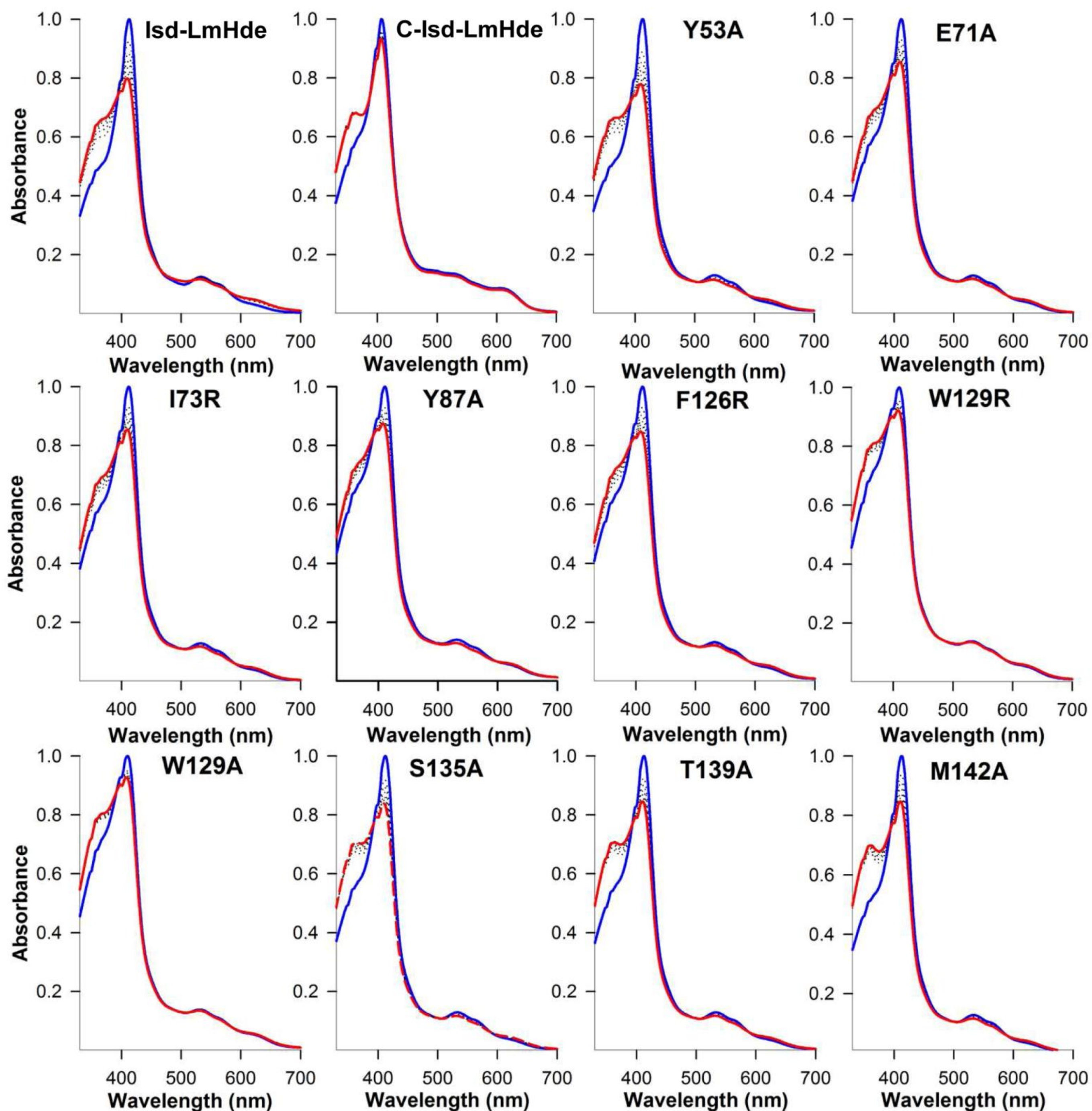

Figure S2. Heme-degrading activity of Isd-LmHde and its mutants. Absorption spectra of 10 μM hemin-protein complex in the presence of ascorbic acid and catalase at a molar ratio

of 0.5:1.0 (catalase:hemoprotein). Spectra were taken immediately before addition of ascorbic acid and then every 5 min. The name of each mutant was denoted above each set of spectra. The spectrum before the addition of ascorbic acid is shown as a solid blue line (——), spectra of the intermediate time points (every 5 min) after the addition of ascorbic acid are shown as dotted lines (……), and the spectrum at 30 min is shown as solid red line (——). The maximum absorption values of Isd-LmHde, C-Isd-LmHde, Y53A, E71A, I73R, Y87A, F126R, W129R, W129A, S135A, T139A, and M142A are 1.27, 1.09, 1.14, 1.17, 1.22, 0.99, 1.25, 1.07, 1.12, 1.30, 1.34, and 1.23, respectively. These maximum absorption values were normalized to 1.0 for clearer comparison.

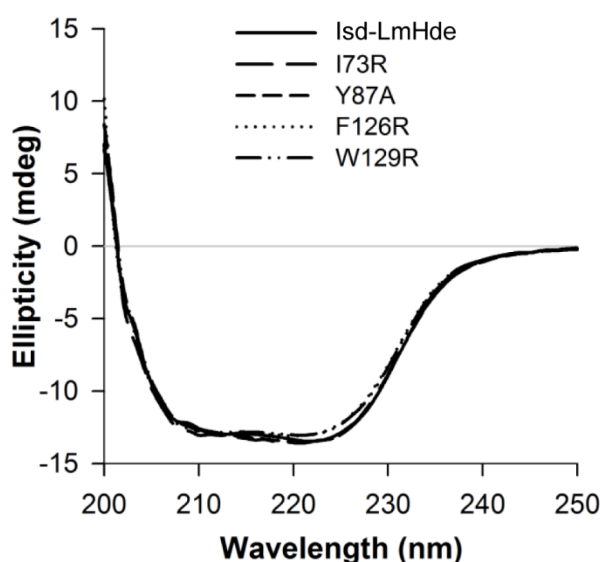

Figure S3. CD spectra of the wild-type and the mutant Isd-LmHde proteins. The protein concentrations were 10 μM in a buffer containing 50 mM Tris pH 8.0 and 150 mM NaCl. Each spectrum represents the average of three scans. CD spectra of wild-type protein, I73R mutant, Y87A mutant, F126R mutant, and W129R mutant are shown as a solid line (——), a long dashed line (— — —), a short dashed line (- - - -), a dotted line (……), and a dash-dot-dot line (—••), respectively, and are also indicated in the figure. The spectrum of W129A is as same as that of W129R and the spectra of E71A, S135A, T139A, and M142A are as same as

that of I73R, thus they are not presented in the figure. The CD spectra of mutant proteins are very similar to the spectrum of the wild-type protein, and thus those are not clearly identified.

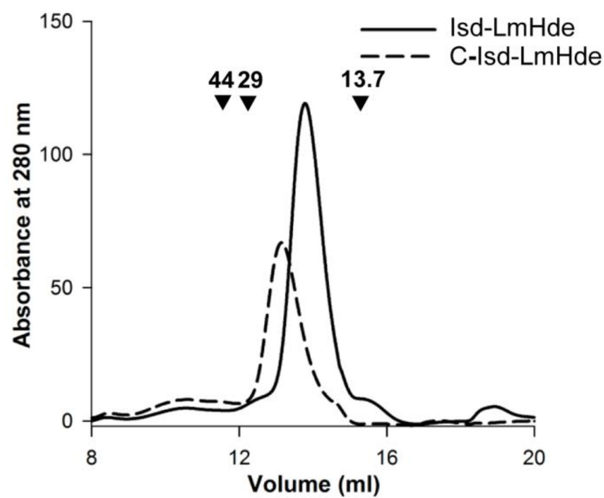

Figure S4. Size-exclusion chromatography profile of full-length Isd-LmHde (——) and the C-Isd-LmHde (- - - -) with molecular weight markers indicated above black triangles: ovalbumin (44kDa), carbonic anhydrase (29kDa), and ribonuclease A (13.7kDa).

Table S1. Bacteria strains and plasmids used in this study.

Strain or plasmid	Genotype or description	Reference or source
Strains		
E. coil BL21(DE3)	protein expression host	Novagen, Madison, WI, USA
E. coli DH5α	cloning host	Novagen, Madison, WI, USA
E. coli B834 (DE3)	protein expression host (Methionine-auxotrophic strain)	Novagen, Madison, WI, USA
Plasmids		
pvFT1s	expression vector	Korea Patent 1020050051893

Table S2. Primers used in this study (restriction enzyme sites are underlined).

Primer name	Sequence 5'- 3'	Purpose
isd-LmHde-F	GCGCGCGGATCCATGAAAAAAGTATTTATCACA	Cloning of *isd-LmHde* gene
isd-LmHde-R	CGGCCGCTCGAGCTATTTAGCATCATGGGAAAA	
C-isd-LmHde-F	ATATATGGATCCGCTGAGTATCGAGTATT	Cloning of C-*isd-LmHde* gene
C-isd-LmHde-R	ATATATCTCGAGCTATTTAGCATCATGGG	
hmoB-F	GCGCGCGGATCCATGAAGGTTTATATTACATAT	Cloning of *hmoB* gene
hmoB-R	GCGCGCCTCGAGCTATTCGACAGCGAAATATGT	
isdG-F	GATCGGATCCATGAAATTTATGGCAGAAAATAG	Cloning of *isdG* gene
isdG-R	TCGACTCGAGTTATTTCATGTAACTATAGCCTA	
E71A-F	GGAGTAGTCGTCTTTGCATATATCCACCTCCGC	Mutation of E71A
E71A-R	CGCGGAGGTGGATATATGCAAAGACGACTACT	
I73R-F	GGTTTTGGAGTAGTCGTCTTTGAATATAGACAC CTCCGCGATG	Mutation of I73R
I73R-R	CATCGCGGAGGTGTCTATATTCAAAGACGACTA CTCCAAAACC	
Y87A-F	AGAAATCCCTATTTTCTTGCAAATGGCTCAACG AGCTAGTCTTCATTTTAGT	Mutation of Y87A
Y87A-R	ACTAAAATGAAGACTAGCTCGTTGAGCCATTTG CAAGAAAATAGGGATTTCT	

F126R-F F126R-R	CCTTTTGGGATTCTGAAGTATTCCGTCATGACTG GAAAAAATCACC GGTGATTTTTTCCAGTCATGACGGAATACTTCA GAATCCCAAAAGG	Mutation of F126R
W129R-F W129R-R	GATTCTGAAGTATTCTTTCATGACCGGAAAAAA TCACCTTTATCAAAAG CTTTTGATAAAGGTGATTTTTTCCGGTCATGAA AGAATACTTCAGAATC	Mutation of W129R
W129A-F W129A-R	GGGATTCTGAAGTATTCTTTCATGACGCGAAAA AATCACCTTTATCAAAAG CTTTTGATAAAGGTGATTTTTCGCGTCATGAA AGAATACTTCAGAATCCC	Mutation of W129A
S135A-F S135A-R	GACTGGAAAAAATCACCTTTAGCAAAAGAAAT TACAAATATCATGAG CTCATGATATTTGTAATTTCTTTTGCTAAAGGTG ATTTTTTCCAGTC	Mutation of S135A
T139A-F T139A-R	CACCTTTATCAAAAGAAATTGCAAATATCATGA GAAAAAATAATACTC GAGTATTATTTTTTCTCATGATATTTGCAATTTC TTTTGATAAAGGTG	Mutation of T139A
M142A-F M142A-R	CACCTTTATCAAAAGAAATTACAAATATCGCGA GAAAAAATAATACTCAATCTGGATTCTC GAGAATCCAGATTGAGTATTATTTTTTCTCGCG ATATTTGTAATTTCTTTTGATAAAGGTG	Mutation of M142A

Table S3. Kd values for heme binding to the wild-type and the mutant Isd-LmHde proteins

Protein (Isd-LmHde)	K_d value (μM)
Wild-type	9.75 ± 0.36
I73R	9.29 ± 0.58
Y87A	10.15 ± 0.57
F126R	7.29 ± 0.36
W129R	8.36 ± 0.29