Experimental procedures

Electronic Absorption Spectroscopy- All absorption spectra of the purified EfeB-heme complex and its mutants were measured at ambient temperature in 10 mM Tris-Cl buffer pH8.0, containing 10 μM of purified heme-bound proteins. By comparison, the spectra changes were also recorded for heme-bound proteins with addition of 500 μM H_2O_2.

The protein sample was divided into two aliquots to test whether O_2 acts as ligand of the EfeB-heme complex. The first sample was treated with 500 μM sodium dithionite, and then the spectra changes were recorded before and after flushing with CO. For the second sample, the soret band changes were measured directly before and after CO treatments.

Results

In order to examine whether O2 is the ligand of the EfeB-heme complex, the EfeB-heme sample in the reduced state was flushed with CO, the soret band was shifted from 432 nm to 420 nm, indicating that CO is a ligand of ferrous-heme bound EfeB. However, the EfeB-heme in oxidized state has the soret peak at 406 nm with or without exposure to CO, indicating that EfeB with ferric-heme does not bind CO. These data suggest that EfeB-heme complex in oxidized state does not bind O2 as ligand in solution, since the ligand of O2 generally can be easily replaced with CO.

The soret band for the purified EfeB-heme complex is 406 nm, however, the soret band of heme-bound D235N mutant is 412 nm. This spectral difference may reflect local chemical environment change in the heme-binding region of the D235N mutant. When the wt and D235N proteins reacted with H_2O_2 about 10-30 seconds at room temperature, the soret bands were all shifted to 417 nm.

Figures

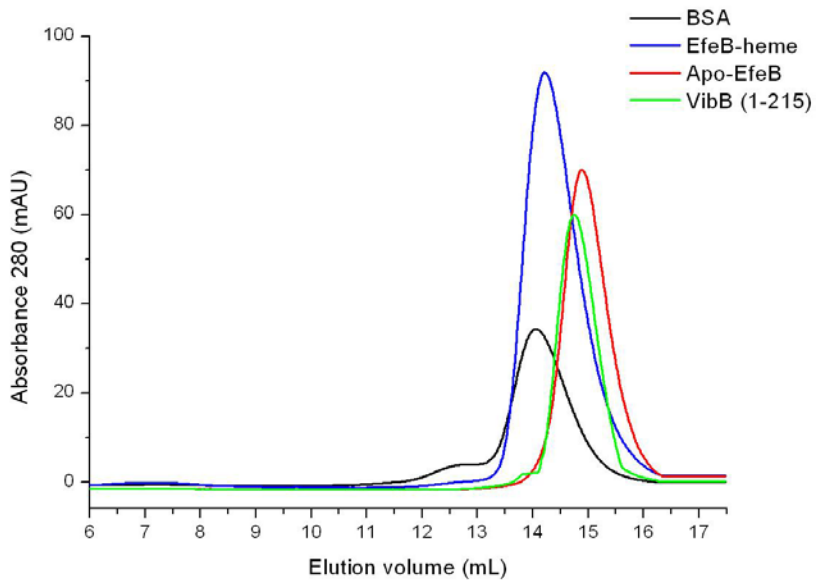

Figure S1. Chromatographic analysis of heme-bound EfeB and apo-EfeB

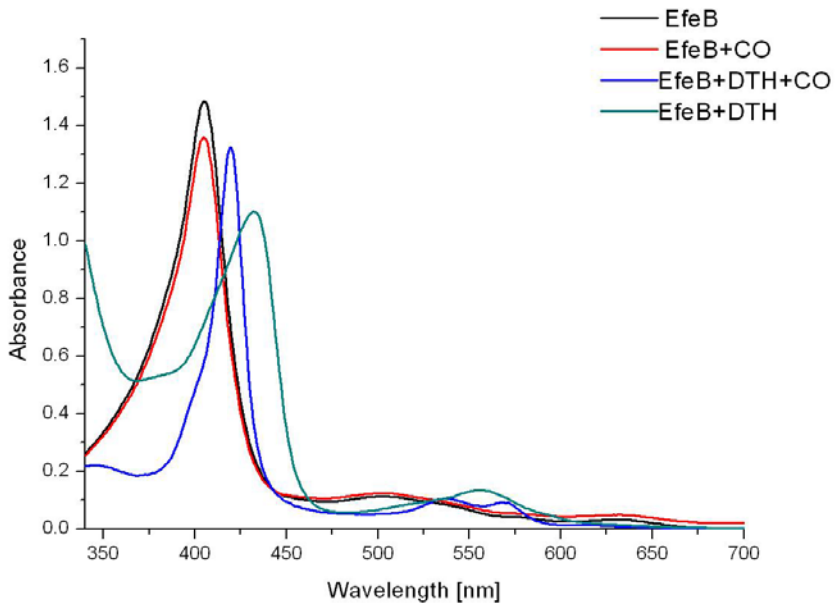

Figure S2 Characterization of EfeB-heme complex using UV-visible spectroscopy

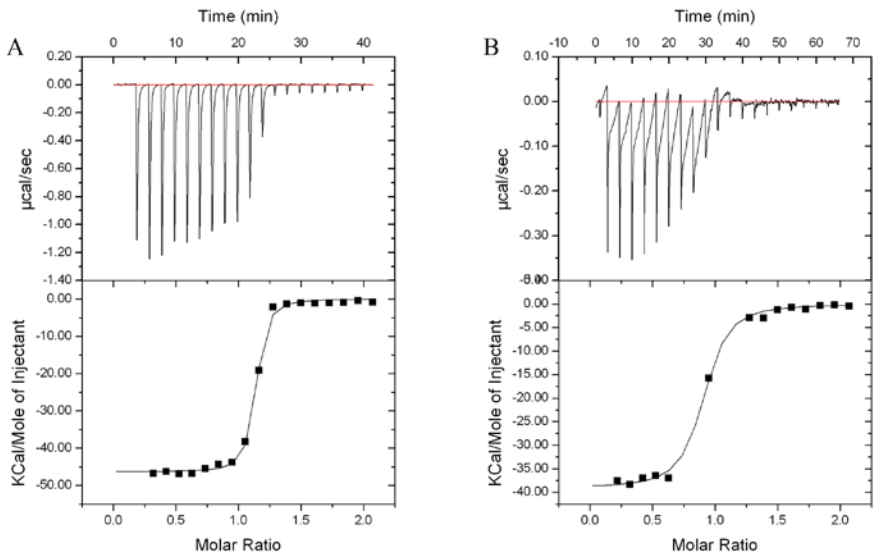

Figure S3. ITC data of hemin titrates to apo-EfeB (A) and hemin titrates to EfeB-PPIX complex (B)

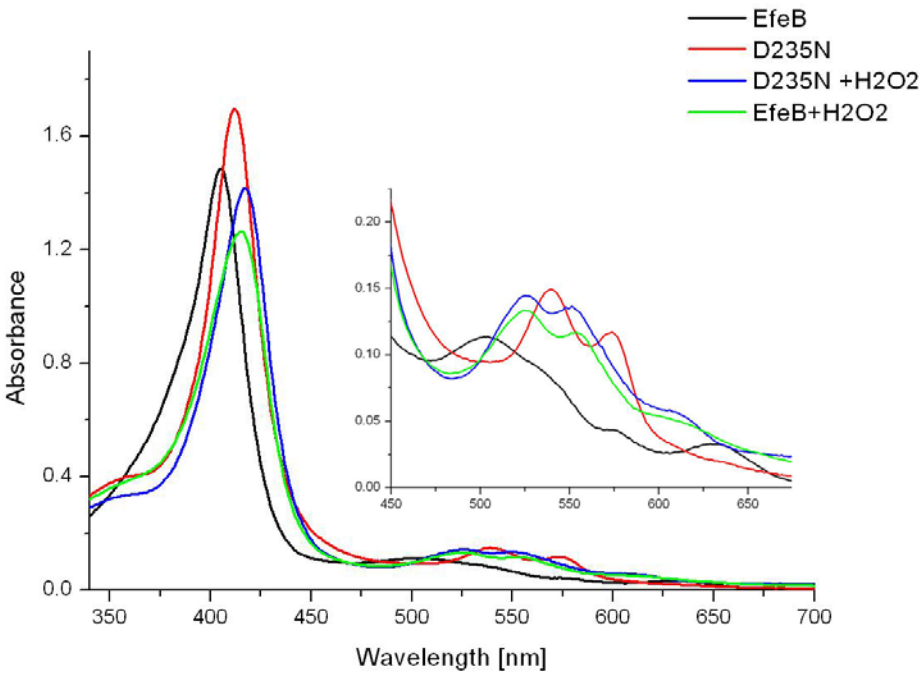

Figure S4. Absorption spectra of purified heme-bound EfeB and the mutant D235N and their reaction with H_2O_2