

Supporting Information

Supporting figure legend

SI Figure 1

Linear dependence of Trp fluorescence on the amount of reconstituted PpsR-heme.

SI Figure 2

PpsR binds to heme. (A) Identification of cofactor with PpsR-flag. 10 μ M PpsR was incubated with 5 μ M heme, Zinc porphyrin (ZnPP) or protoporphyrin IX (PPIX). (B) PpsR binds to hemin-agarose. Purified apo-PpsR was incubated with 10 μ l of hemin-agarose resin (Sigma) for at least 1 hour at room temperature. The hemin-agarose was then washed with 1 ml buffer (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5% glycerol) three times. The washing buffer and hemin-agarose were then directly applied to SDS-PAGE. From left to right, lane 1: purified apo-PpsR. Lane 2: wash buffer 1. Lane 3: wash buffer 2. Lane 4: hemin-agarose. (C) Purification of PpsR using SUMO system. From left to right, lane 1: SUMO-PpsR after His-tag column. Lane 2: Cleavage of SUMO-PpsR. Lane 3: separated tag-less PpsR. (D) The spectrum of deoxy-PpsR-heme. 5 μ M heme were incubated with 10 μ M PpsR for at least 20 minutes, then reduced with 5 mM dithionite. All samples were completely degassed in advance and the procedure was performed in anaerobic hood and using flow-stop cuvette. (E) UV-visible spectrum of PpsR-heme-CO: 3.3 μ M heme were incubated with 8.7 μ M PpsR for at least 20 minutes, then reduced with 5 mM dithionite. Fresh carbon-monoxide was bubbled into sample and the spectrum was recorded immediately.

SI Figure 3

Permutation assay of *puc* promoter region. (A) Probes used for permutation assays. The diagram was drawn in scale except the part of *pucB* gene outlined in dash lines. (B) Gel mobility shift assays with probe one to five. (C) Electrophoresis patterns of probe one to five. In each experiment conditions, the electrophoresis distance of the probe was normalized to probe three which has the PpsR binding site in the middle. The X axis represented the number of base pairs that separate the PpsR binding site and the right end of the probe.

SI Figure 4

Heme effect on PpsR-DNA binding. (A) The effect of heme concentration on PpsR-*puc* binding. 8.7 nM *puc* DNA was used to bind 8.7 μ M PpsR with different concentration of heme. (B) The effect of heme concentration on PpsR-*hemE* binding. 8.7 nM *hemE* DNA was used to bind 8.7 μ M PpsR with different concentration of heme. (C) The effect of chemicals on PpsR-DNA interactions. (From left to right) Lane 1: 10 nM *hemE*. Lane 2: 10 nM *hemE* was incubated with 5 μ M PpsR for at least 20 minutes. Lane 3 ~ 8: the same reaction as lane 2 was performed in the presence of : lane 3, 5 μ M FeCl₃; lane 4, 5 μ M zinc porphyrin; lane 5, 5 μ M protoporphyrin; lane 6, 2.5 μ M heme; lane 7, 5 μ M heme; lane 8, 10 μ M heme.

SI Figure 5

PpsR regulated photosynthesis and tetrapyrrole biosynthesis genes. Identified PpsR regulated genes are labeled by the squares next to the gene names, and the numbers represent the number of base pairs that separate two PpsR-binding sites. The open squares represent the genes regulated by PpsR with no PpsR-binding site identified. *: multiple genes under same promoter. #: *hemC* and *hemE* share part of their promoters.

SI Figure 6

Heme effect on PpsR regulated genes. After 10 minutes of starvation in Siström's minimal medium, the samples were taken and used as the standard for normalization. 25 μ M of heme was added into the culture after 20 minutes of starvation and samples were taken at different time points, measured with qPCR and normalized. Data shown were mean \pm SEM. (A) *puc* promoter. (B) *hemE* promoter. (C) *bchC* promoter. (D) *crtA* promoter.

Supporting Tables

SI Table 1

Primer sequences for probe synthesis

<i>puc</i>	GGACAGGCAGCGTCAATTTCCCGC, TAAATCGACGGTTTGCGTGTAGGGCCTG
<i>hemE</i>	TGTCCTTCATCGAATGGACCGCGATGTCGA, CAAGCTGAGCATGGGATGTCAATATGGGGA

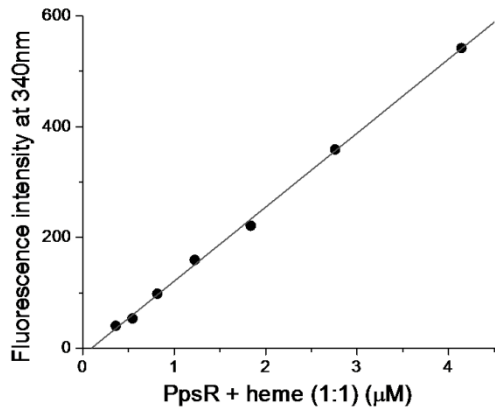
SI Table 2

Primer sequences for qPCR analysis

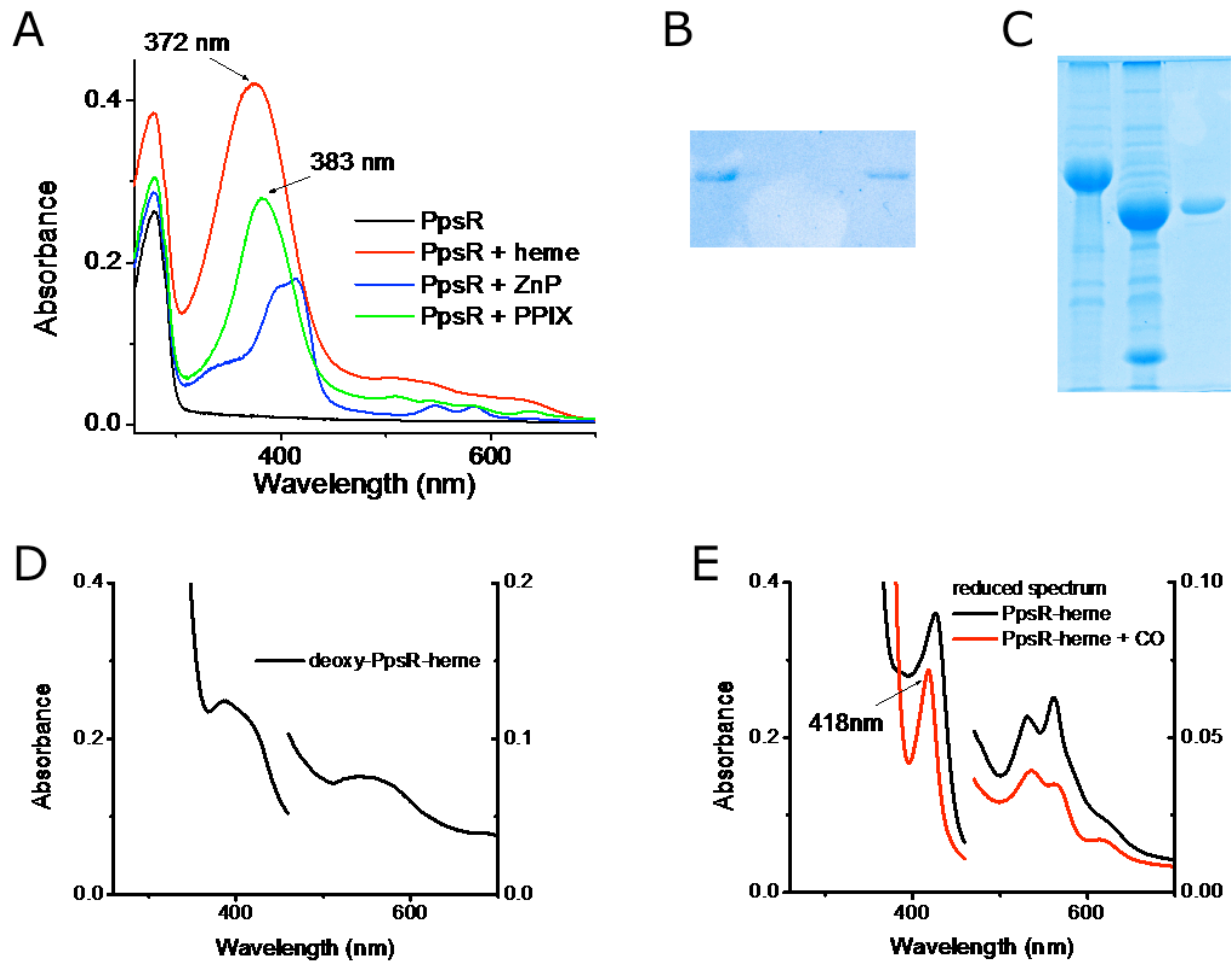
<i>rpoZ</i>	ATCGCGGAAGAGACCCAGAG, GAGCAGCGCCATCTGATCCT
<i>puc</i>	GGCAAATCTGGCTCGTGGT, GGTGGTGGTCGTCAGCACAG
<i>hemE</i>	GACAACCTTCATCGGCTTTG, GTCGGTGTCTTCAGCTTGT
<i>bchC</i>	ACCTTGGCCTTCAGACCATC, ATCTGCCCGGTGTAGAACAG
<i>crtA</i>	TCACGCTCAGTATCTTCCGGTTC, CCAGCTTGTTTGCGGTCATCT

Supporting Figures

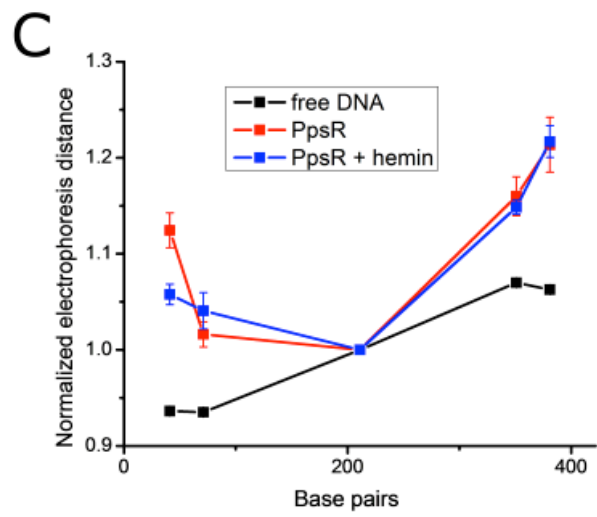
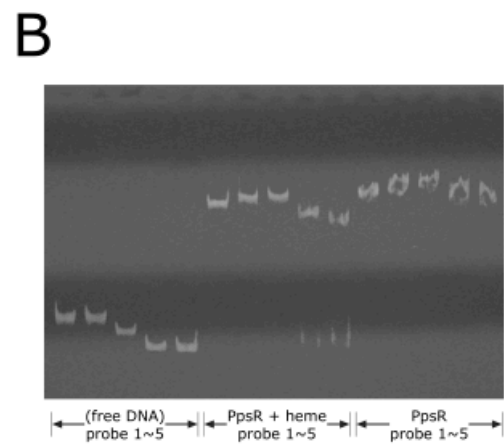
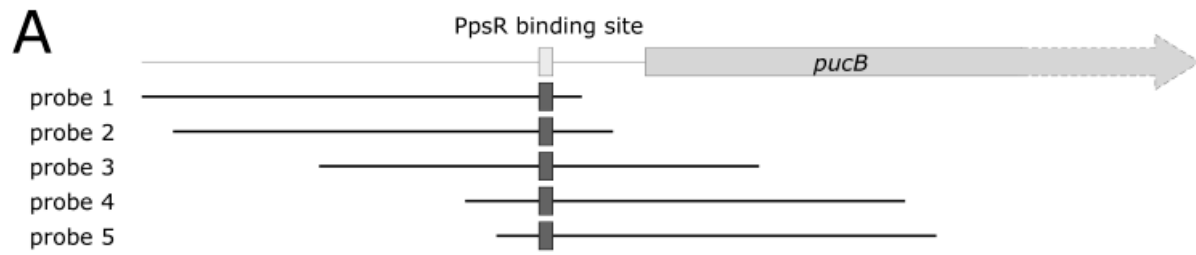
SI Figure 1



SI Figure 2

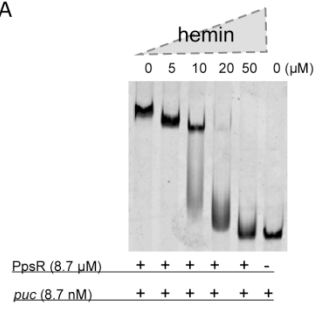


SI Figure 3

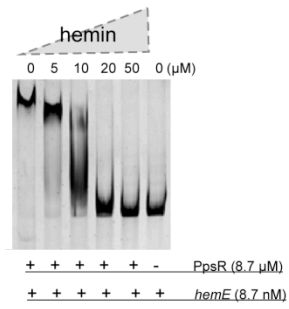


SI Figure 4

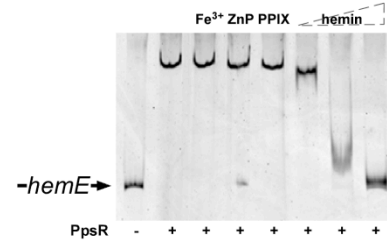
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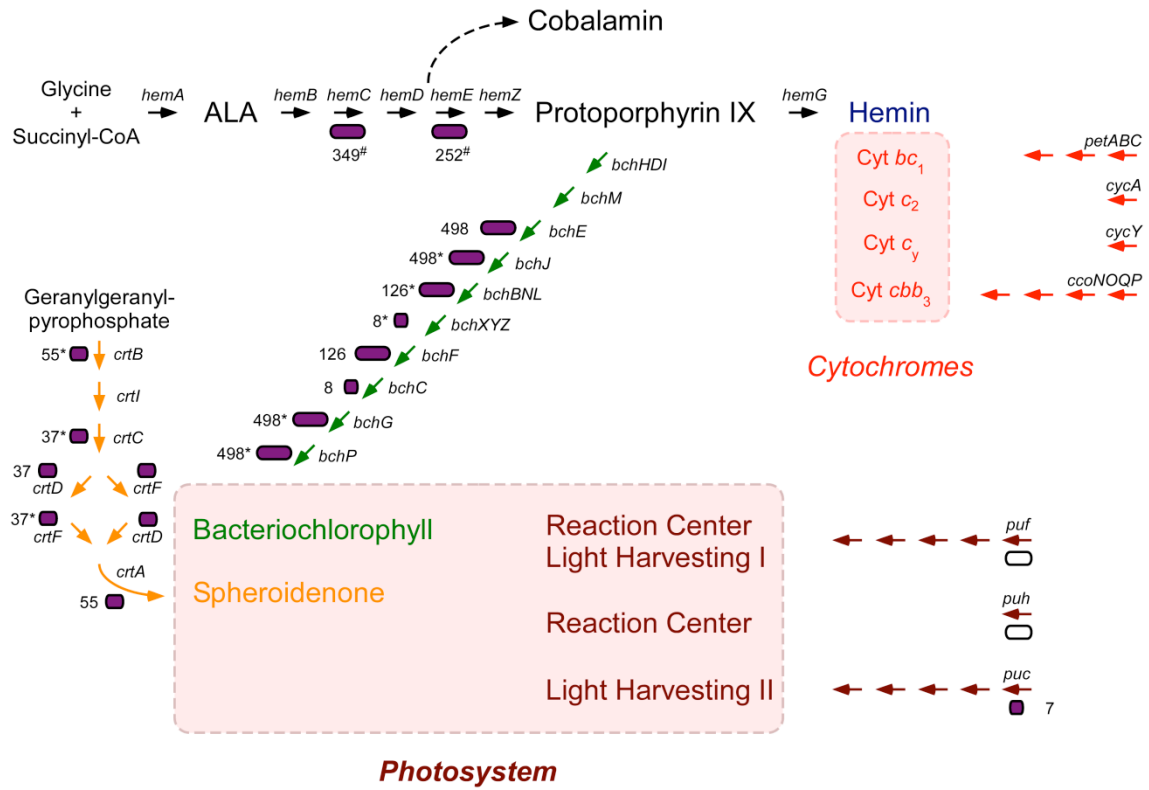
B



C

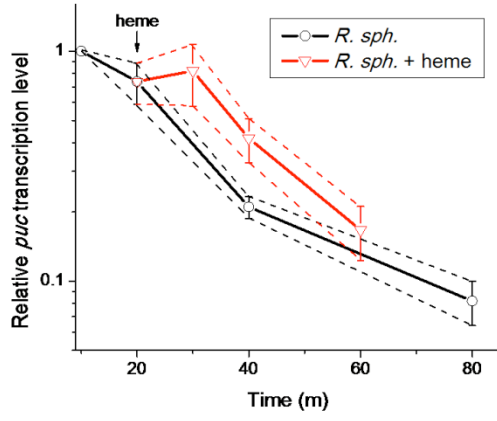


SI Figure 5

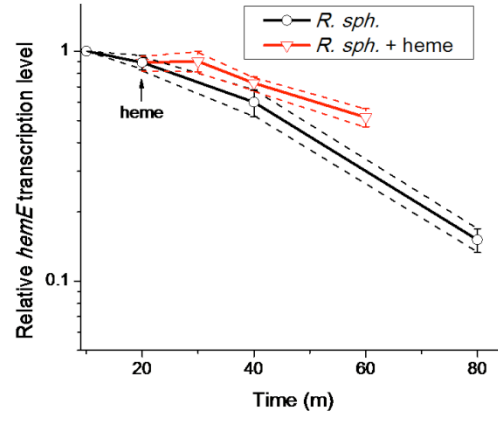


SI Figure 6

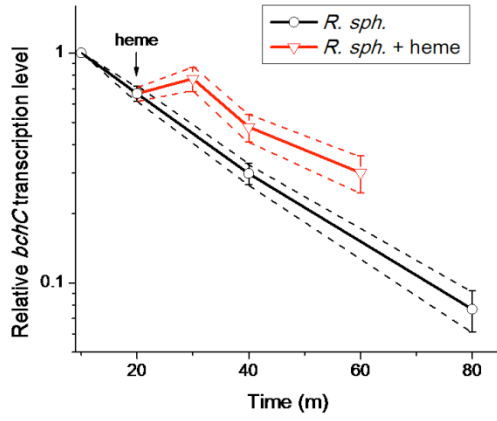
A



B



C



D

