Stationary Phase EPR Spectroscopy for Monitoring Membrane Protein Refolding by Conformational Response

Supporting Information: Figures S1–S3

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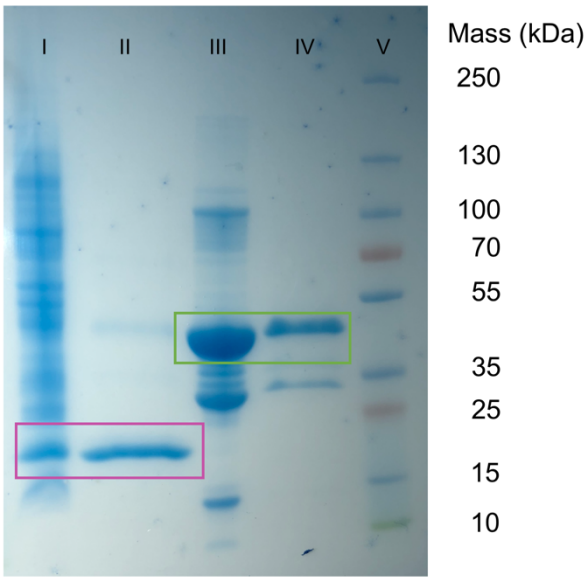

Figure S1. SDS–PAGE of HmbRI (D94N/R172C mutant; boxed in purple) and GST–HmbRI (D94N/R172C mutant; boxed in green). Lane I: Isolated *E. coli* membranes with overexpressed HmbRI mutant. Lane II: HmbRI mutant eluted from Ni-NTA agarose resin. Lane III: Isolated inclusion bodies with overexpressed GST–HmbopI mutant. Lane IV: GST–HmbRI mutant refolded in the presence of all-*trans*-retinal on and eluted from Ni-NTA agarose resin. Lane V: Molecular weight ladder. When the reported refolding workflow is performed on HmbRI or GST–HmbRI mutants lacking cysteine residues, no noticeable nitroxide EPR signal is observed after treatment with MTSSL. Hence, the minor impurities in Lanes II and IV still present after Ni-NTA purification do not contribute to EPR spectra presented in the main text.

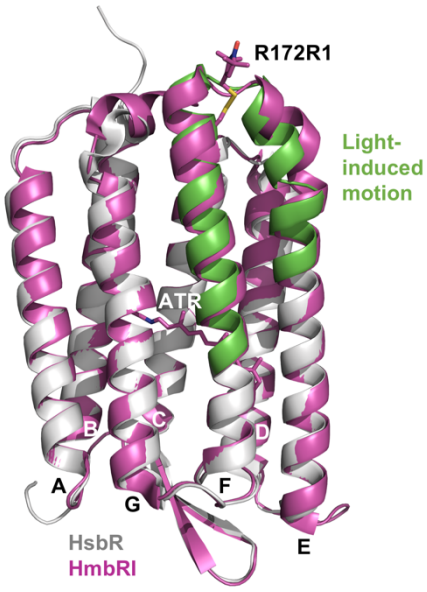

Figure S2. Structural overlay of HsbR (PDB ID: 5B6V; grey) and HmbRI (PDB ID: 4PXK; purple). Green region of HsbR helices E and F undergo conformational change upon illumination (see Results section of main text). R172 in the equivalent region of HmbRI was chosen as the site for spin labeling. The nitroxide spin-labeled side chain is modeled onto site R172 of HmbRI (R172R1).

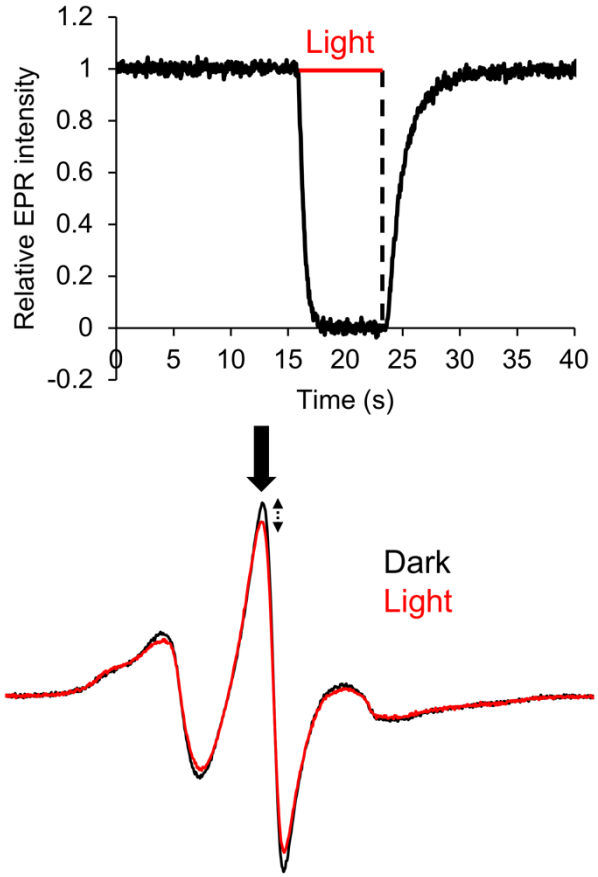

Figure S3: Top: Time course of EPR spectrum of trimeric HmbRI D94N/R172R1 recorded at single field upon illumination (light on for a period indicated by the red bar) with >500 nm wavelength visible light and darkening (light off). Bottom: The static field position is indicated by black arrow above the EPR field sweep. The EPR spectral lineshape changes induced by light are reversible upon re-incubation in the dark.