Supplementary Information

Resonance Raman view of the active site architecture in bacterial DyP-type peroxidases

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Enzyme purification

The DyPs were produced and purified as previously reported.[1-5] Briefly, all enzymes were heterologously overexpressed in *E. coli*. The recombinant strains were grown in either rich[1-5] or minimal media[4]. Growth media for PpDyP and BsDyP was supplemented with 15 μM hemin.[3] After cell disruption and debris removal, the protein extracts were loaded onto affinity collumns[1-2,4-5] (HisTrap) or, in PpDyP and BsDyP purification, onto ionic exchange columns[3] (Q-Sepharose and SP-Sepharose, respectively). The enzymes were then eluted using appropriate buffer systems.

Resonance Raman (RR) spectroscopic measurements

The RR spectra of Cbo, Tfu and DrDyPs were measured as previously described.[1] For room temperature experiments, ca. 80 μL of 100 - 200 μM enzymes in buffer solutions at different pH values (3.5 - 10) were placed in a rotating cuvette (Hellma) to prevent prolonged exposure to laser irradiation. Low temperature measurements were performed using ca. 2 μL of the same sample placed in a microscope stage (Linkham THMS 600) cooled to the desired temperature with liquid N_2. The spectra were acquired in the backscattering geometry using a confocal microscope (Olympus 20× objective) coupled to a Raman spectrometer (Jobin Yvon LabRaman 800 HR) with a CCD detector cooled with liquid N_2. The 413 nm line from a krypton ion laser (Coherent Innova 300c) was used as excitation source. Spectra were accumulated for 20 - 30 s with a laser power of 2 - 3 mW at the sample; typically, 6 to 10 spectra were added to minimize signal-to-noise ratio. Background scattering was removed by subtraction of a polynomial function and the positions and widths of Raman bands were determined by component analysis as described previously.[2]

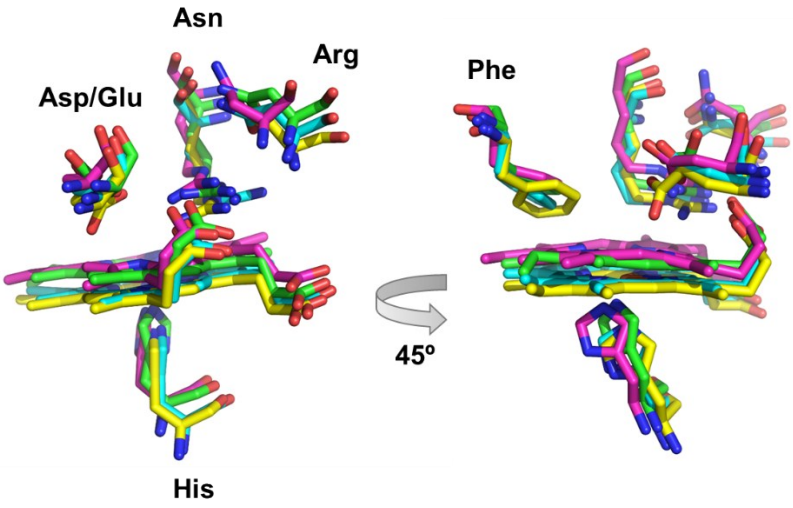

Fig. S1 Detail of the heme binding pocket of Cbo, Tfu, Pp and Vc DyPs indicating the proximal His and the conserved distal residues Asp/Glu, Asn, Arg and Phe.

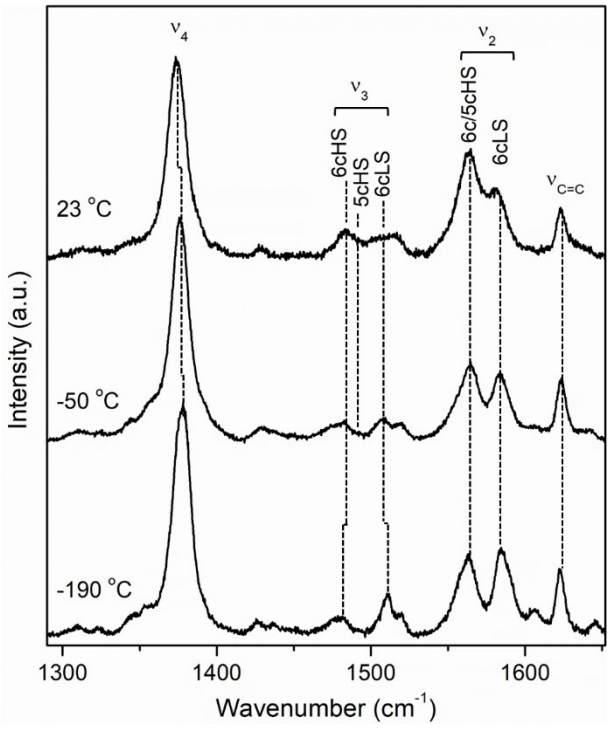

Fig. S2 Temperature dependence of BsDyP spin populations. Spectra were measured at 23, -50 and -190 °C at pH 7.6 with 413 nm laser excitation.

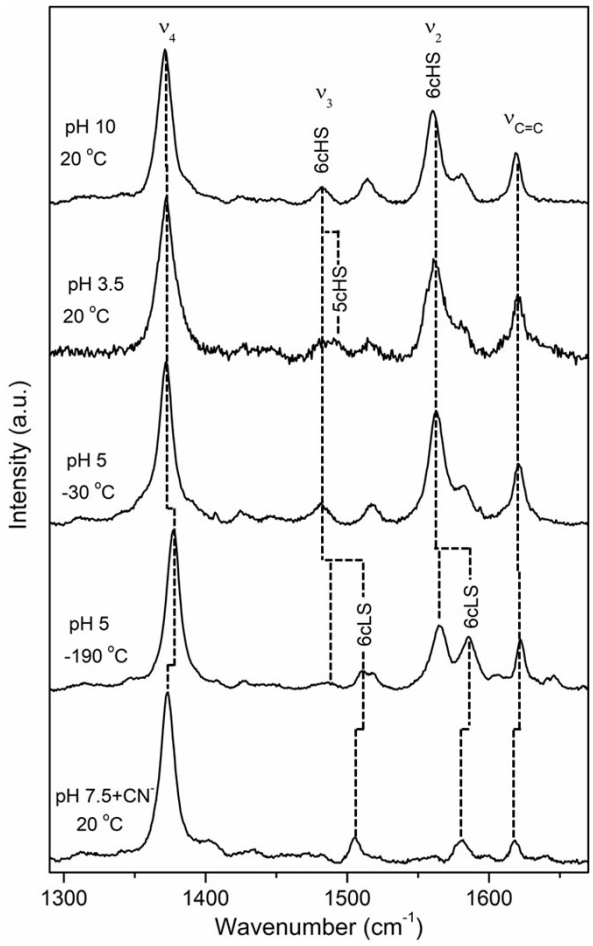

Fig. S3 Temperature and pH dependence of CboDyP spin populations. Spectra were measured at 20 °C in pH 10 and pH 3.5 buffers, at -30 and -190 °C in pH 5 buffer and at 20 °C in pH 7.5 buffer with KCN. Spectra were acquired with 413 nm laser excitation.

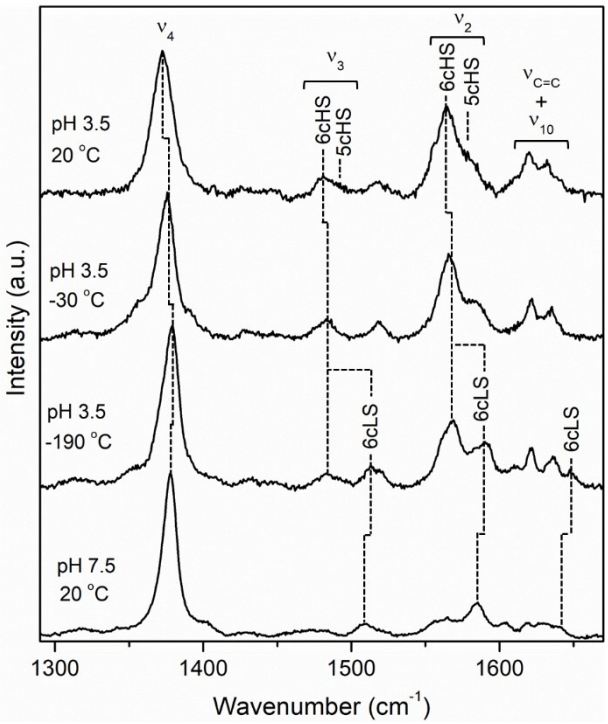

Fig. S4 Temperature and pH dependence of TfuDyP spin populations. Spectra were measured at 20, -80 and -190 °C at pH 3.5 and at 20 °C at pH 7.5. Measurements were obtained with 413 nm laser excitation.

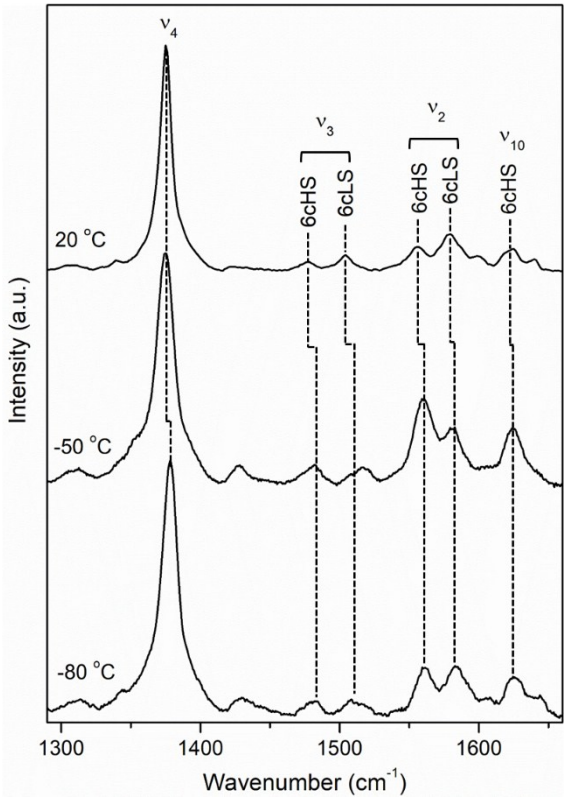

Fig. S5 Temperature dependence of DrDyP spin populations. Spectra were measured at 20, -50 and -80 °C in pH 4.0 buffer, using 413 nm excitation.

References:

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