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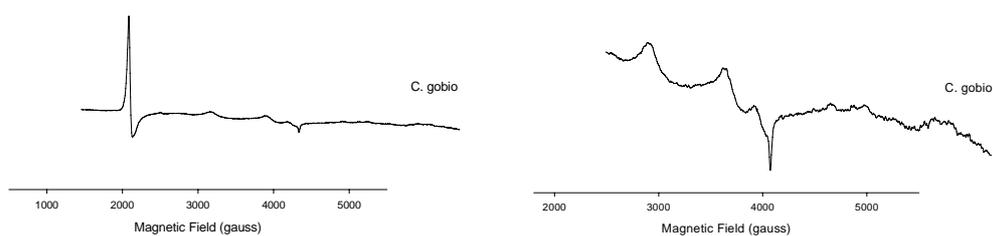
**Supporting Material**

**Correlation between hemichrome stability and Root effect in tetrameric hemoglobins**

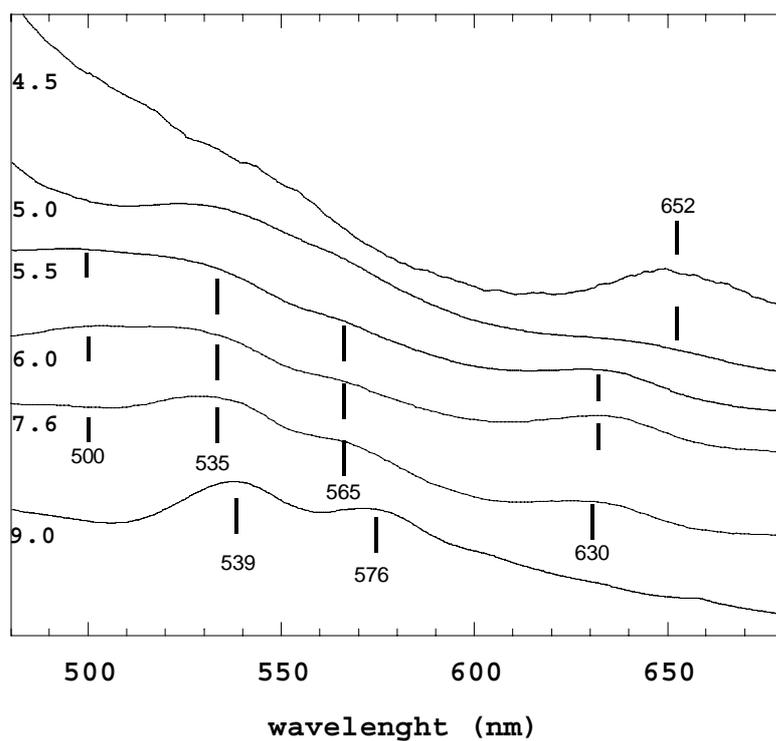
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### Supplementary material

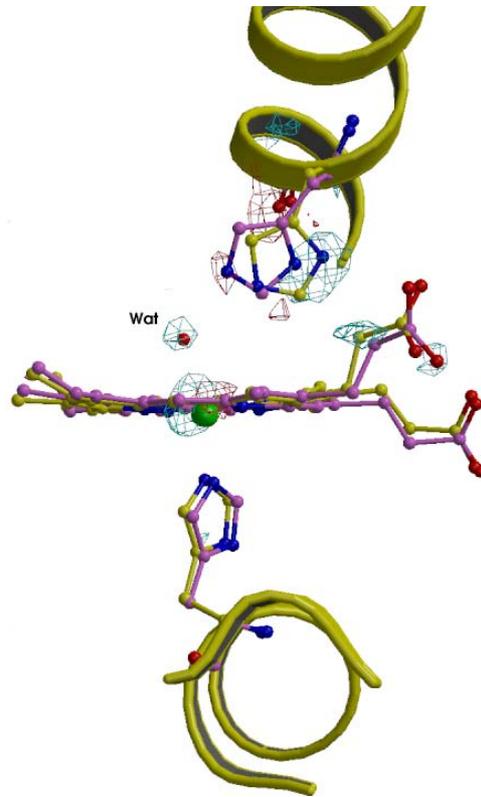
**Fig. S1.** CW-EPR spectra of Hb1Cg. The protein concentration was 0.5 mM tetramer, buffer was 50 mM HEPES pH 7.6. Spectra were recorded at 12 K, microwave frequency of 9.29 GHz, microwave power of 10 mW, modulation frequency of 100 kHz, and modulation amplitude of 5 G. Spectra in the left panel are re-plotted on a  $\times 10$  intensity scale on the right, showing the low-spin signal region.



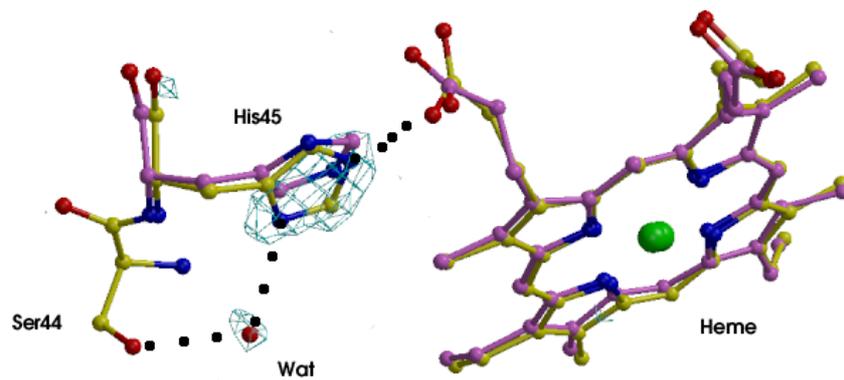
**Fig. S2.** Optical spectra of HbTb as a function of the pH from pH 4.5 up to 9.0. The intensities are normalized to that of the Soret band.



**Fig. S3.** Superimposed structures of pH6-HbTb (yellow) and deo-HbTb (pink). Isomorphous difference map contoured at  $4.0 \sigma$  for the  $\alpha_1$ -heme (A) and His 45 (B). Positive peaks are shown in cyan and negative peaks in red.



A



B