

Ligand Uptake Modulation by Internal Water Molecules and Hydrophobic Cavities in Hemoglobins

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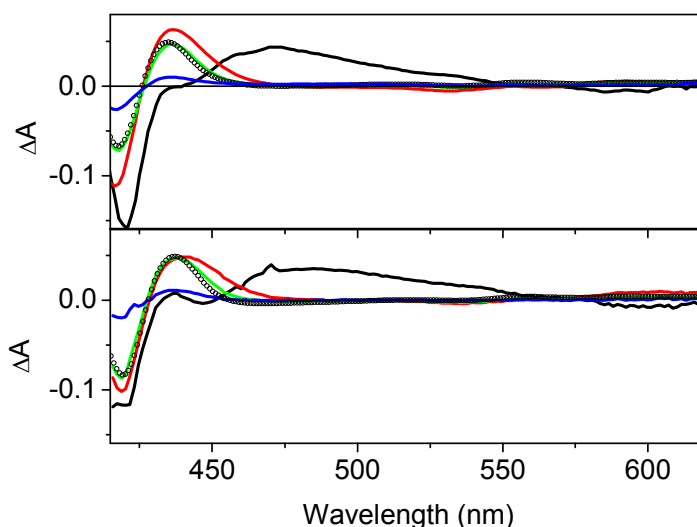


Figure S1. Decay associated spectra (DAS) obtained by the global analysis of the transient data of WG8F (top panel) and 3F *Tf*-trHb (bottom panel). The associated time constants are 0.2 ps (black), 7 ps (red), 400 ps (green) and >>2 ns (blue) for WG8F and 0.2 ps (black), 6 ps (red), 800 ps (green) and >>2 ns (blue) for 3F *Tf*-trHb. For comparison, the properly scaled steady-state absorption difference spectrum (between CO-*Tf*-trHb and 5c-*Tf*-trHb) is reported as open circles.

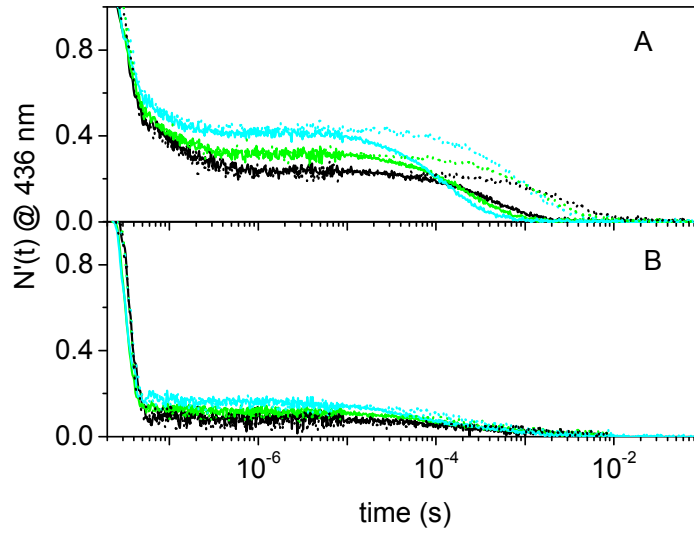


Figure S2. CO rebinding kinetics to WG8F (A) and 3F *Tf*-trHb (B) solutions at $T = 10\text{ }^{\circ}\text{C}$ (black), $T=20\text{ }^{\circ}\text{C}$ (green) and $T=30\text{ }^{\circ}\text{C}$ (cyan), at 1 (solid lines) and 0.1 atm CO (dotted lines). Traces are normalized at the end of nanosecond laser pulse ($N'(t)= \Delta A(t)/ \Delta A(20\text{ ns})$). Protein concentration is $\sim 30\text{ }\mu\text{M}$.

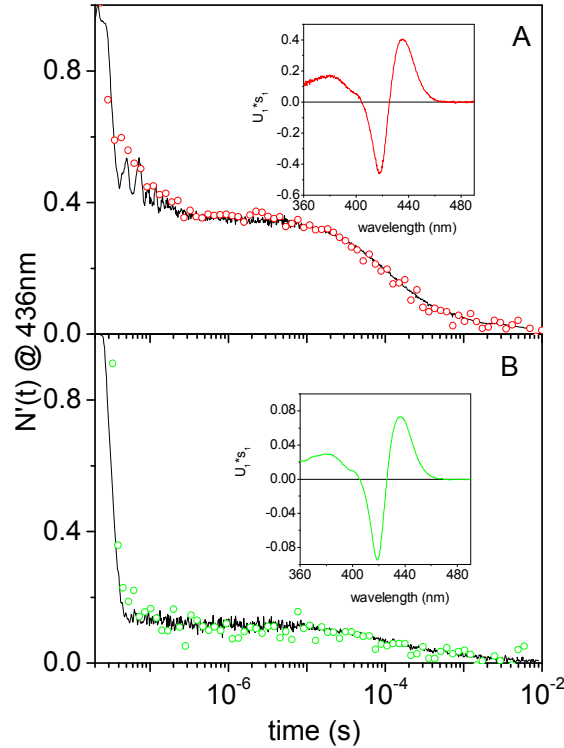


Figure S3. Comparison between the time courses of the amplitudes V_1 (open circles) and the normalized absorbance change measured at 436 nm (in a single wavelength experiment) at the same CO pressure and temperature (panel A: WG8F *Tf*-trHb, panel B: 3F *Tf*-trHb). Traces at 436 nm are normalized at the end of nanosecond laser pulse ($N'(t)=$

$\Delta A(t) / \Delta A(20 \text{ ns})$). The pressure of CO was 1 atm, and the spectra were measured at 20 °C. Inset: first (U_1) component obtained from the SVD analysis on the time-resolved spectra measured. Spectra U_1 are multiplied by the corresponding singular value S_1 (5.3 and 4.5 for WG8F Tf -trHb and 3F Tf -trHb, respectively).