Supplementary Data

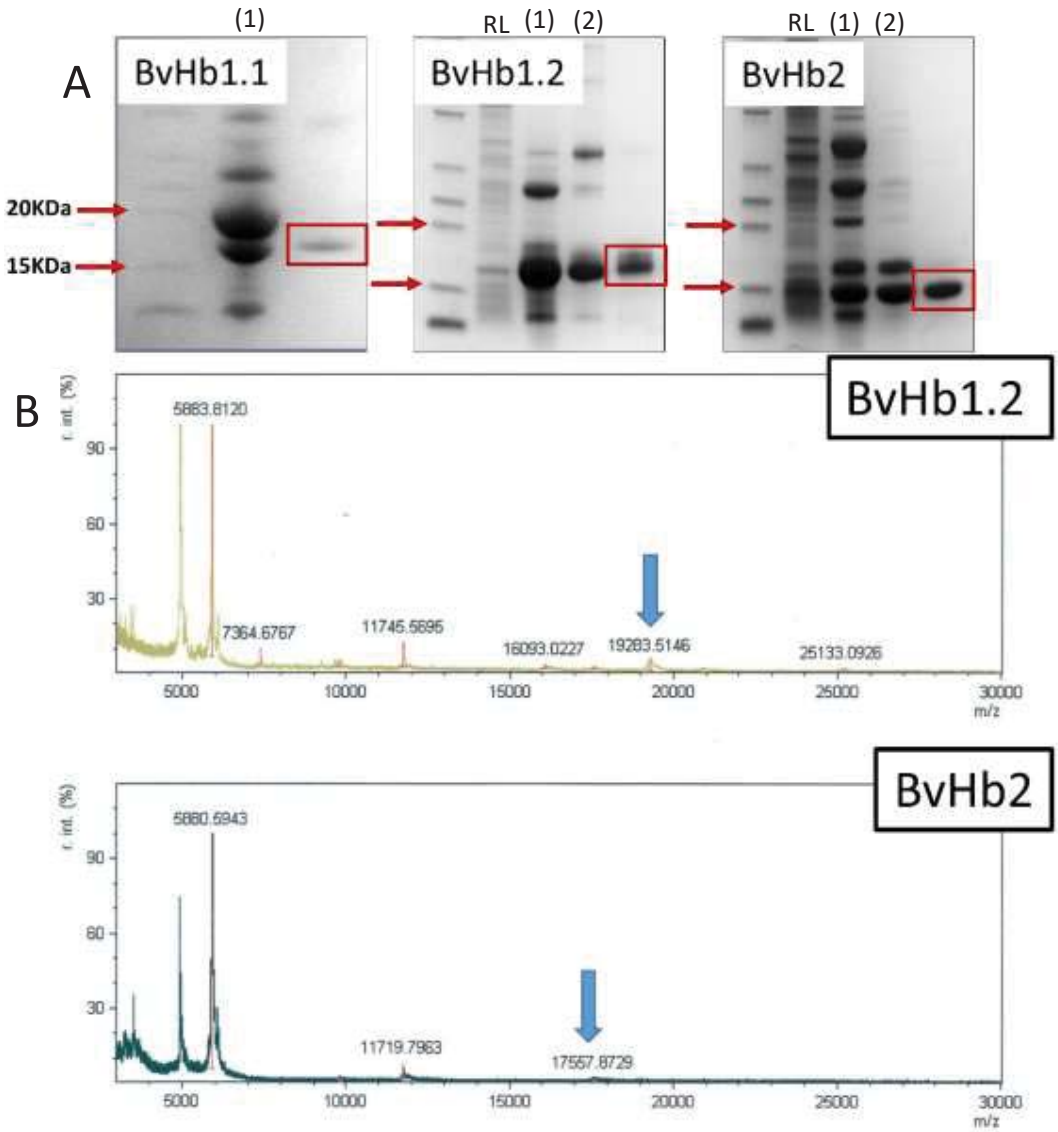

Fig. S1. BvHbs purification and molecular size determination. *(A)* Denaturing SDS-gels where the purification steps (described in Material and Methods) are observed in each lane. The last lane shows in a red square the purified protein used for its characterization. The Raw Lysate (RL) of BvHb1.2 and BvHb2 was also loaded. BvHb1.1 is shown without cTP, traces of a high molecular weight band is observed in the gel and it was also detected when doing gel filtration (Fig. 1A). Upper and lower arrows indicate the protein ladder' size, 20 and 15KDa, respectively. *(B)* Mass spectrometry results for two of the BvHbs, the arrows indicate the size of the monomers in KDa.

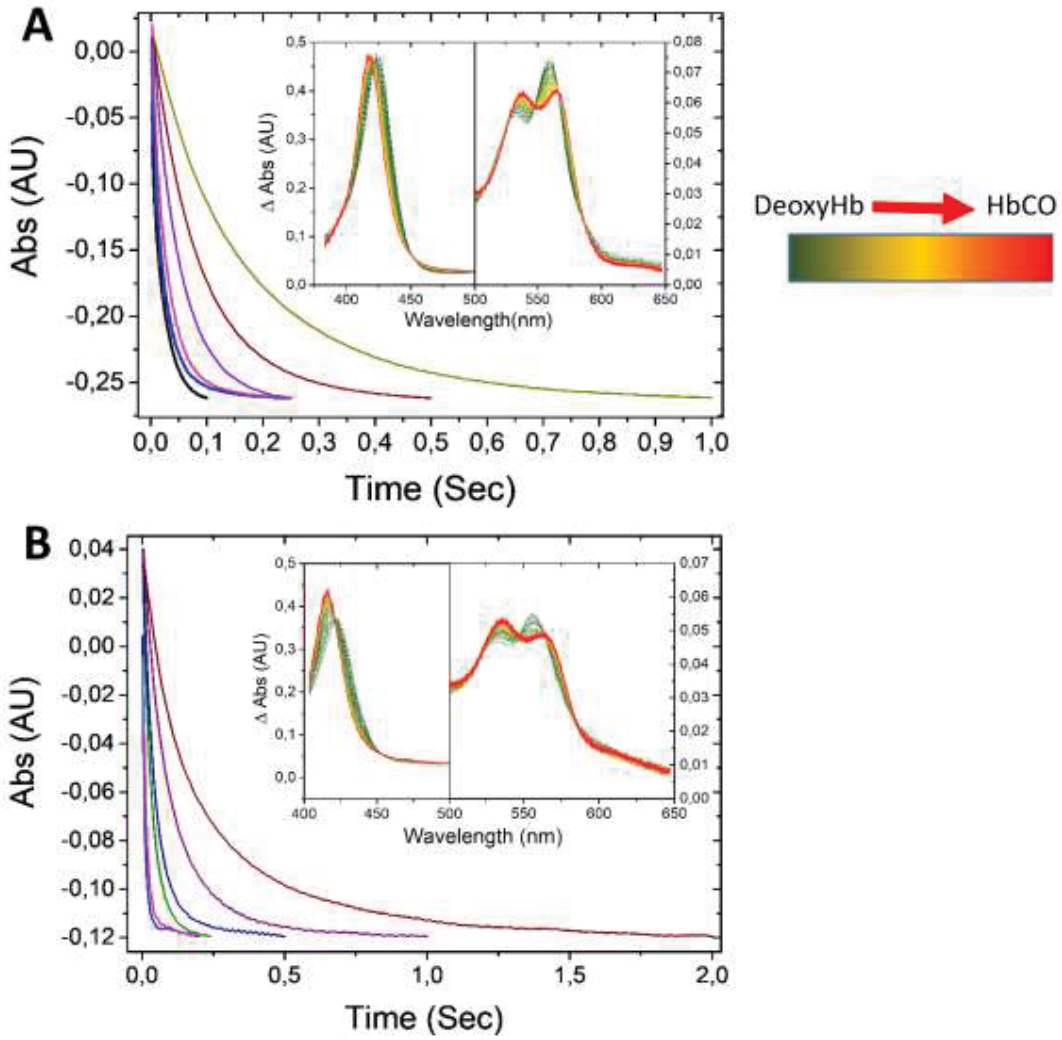

Fig. S2. CO binding to BvHbs by stopped-flow rapid mixing. *(A)* Time courses for CO binding to BvHb1.2 following stopped-flow rapid mixing at different CO concentrations (13, 25, 50, 125, 250, and 500 mM CO, from right to left) monitored at 430 nm. *(B)* Time courses for CO binding to BvHb1.1 following stopped-flow rapid mixing at different CO concentrations (13, 25, 50, 125, 250 mM CO, from right to left). Time courses were monitored at 430 nm. *Insets* in *(A)* and *(B)* show the absorbance spectra associated with the binding of CO to the deoxy-ferrous BvHbs as indicated by the color gradient bar (from green to red).

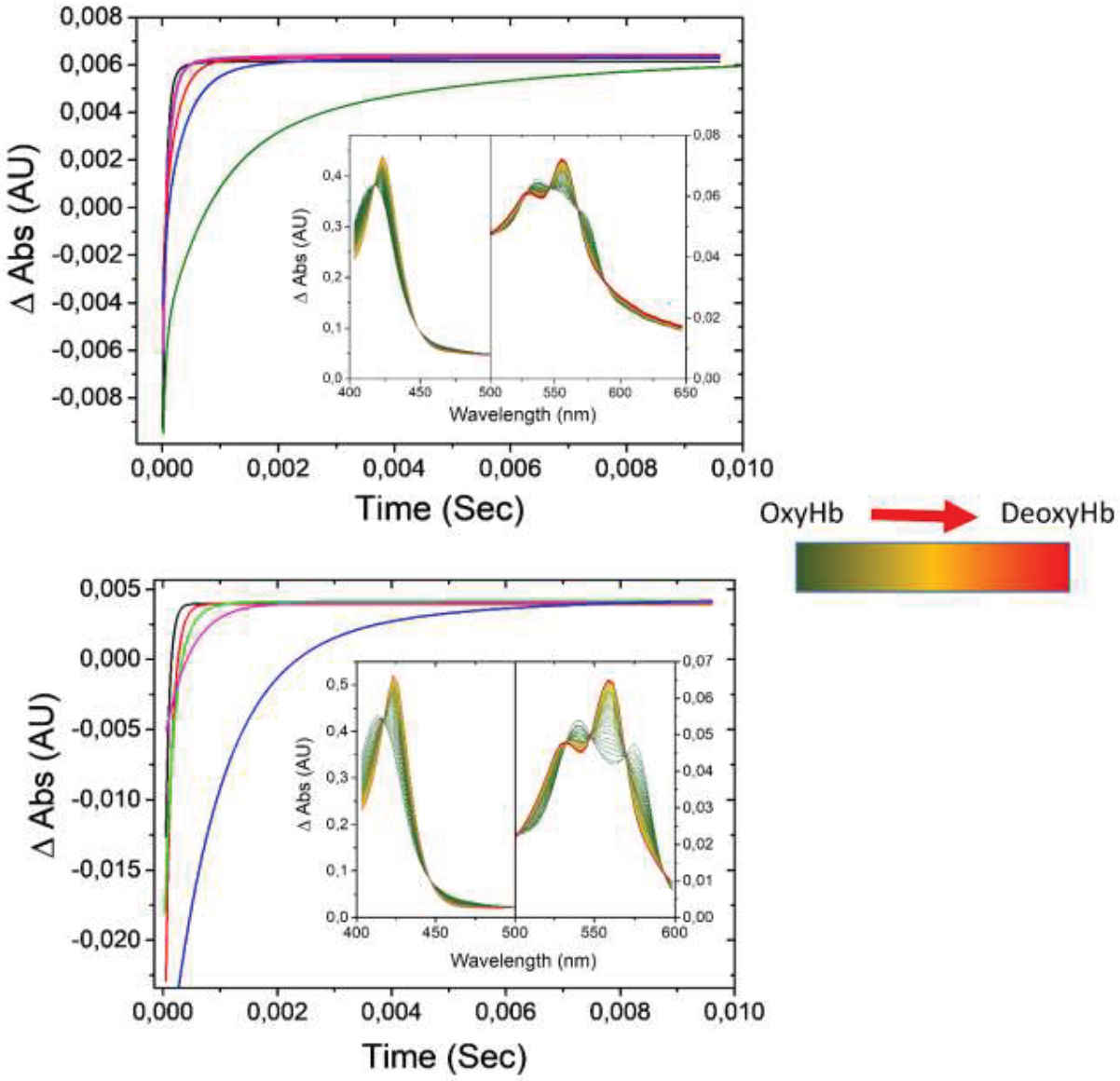

Fig. S3. Oxygen binding and dissociation. Time courses for rebinding of oxygen to BvHb1.1 *(A)* and BvHb1.2 *(B)* after flash photolysis at different oxygen concentrations. From right to left: 5, 31, 62, 125, and 250 µM for BvHb1.1 and 5, 32, 63, 125, 250 µM for BvHb1.2. The time courses were monitored at 410 nm. *Insets*: Absorbance spectra associated with the dissociation of oxygen from BvHb1.1 *(A)* and BvHb1.2 *(B)*. The direction of the spectra change is indicated by the arrow, from oxyHb to deoxyHb.

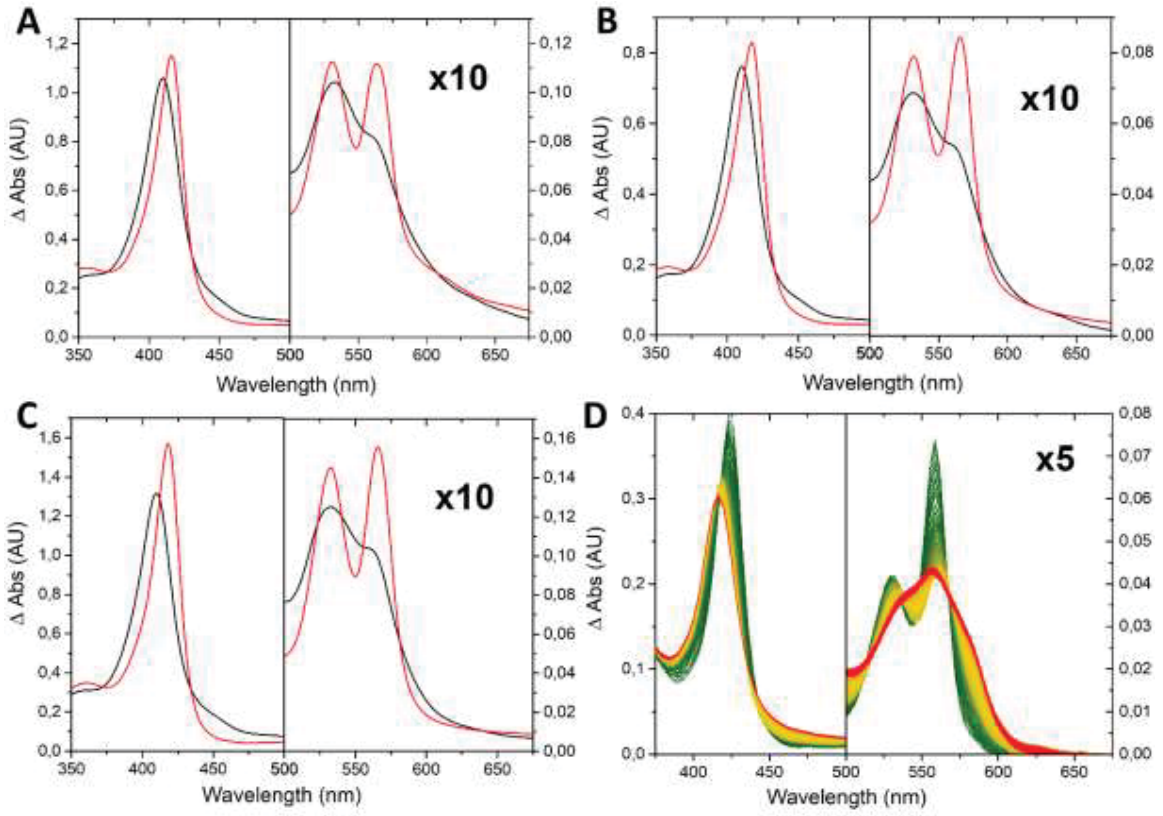

Fig. S4. BvHbs Nitrite reductase activity. *(A-C)* Static spectra of ferric BvHbs (black line) and after binding NO (Fe^{3+}-NO, red line). BvHb1.1, BvHb1.2, and BvHb2 are, respectively, represented in A, B, and C. *(D)* Stopped flow experiment, carried out at a very low dithionite concentration, showing the initial ferrous form of BvHb2 (green) converted to the ferric NO form (yellow) proving a nitrite reductase activity and then to the ferrous NO form (red). The sequence of the reaction goes from dark green to yellow and finally red.