

## Supplementary Information

### *Resonance Raman Spectra*

In the spectra for nHbO<sub>2</sub>, the values for  $\nu_3$  (1507 cm<sup>-1</sup>),  $\nu_{38}$  (1559 cm<sup>-1</sup>),  $\nu_2$  (1580 cm<sup>-1</sup>) and  $\nu_{37}$  (1599 cm<sup>-1</sup>) are typical for hexacoordinated LS heme complexes and correspond to an average C<sub>T</sub>-N distance of 1.989 Å (1). Note that the intensity of  $\gamma_7$  (see Figure 4aA), an out-of-plane mode that is sensitive to the out-of-plane distortion of the heme group, is low. This suggests that the heme group is in a relaxed state, where the iron lies almost completely in the porphyrin plane, which agrees with the above derived porphyrin core size.

In the spectra of all investigated forms of *S. solidissima* nHb, the two vinyl modes ( $\delta(\text{C}_\beta\text{C}_a\text{C}_b)_2$  and  $\delta(\text{C}_\beta\text{C}_a\text{C}_b)_4$ ) coincide in a broad band around 420 cm<sup>-1</sup>. This is in contrast to ferrous deoxy Mb and to MbCO, where these two frequencies are clearly separated from each other (2). This splitting relates to a different influence of the out-of-plane distortions on each of the pyrrole rings, giving rise to a distinct modification of the two vinyl couplings to the porphyrin modes. The coinciding of the two vinyl modes agrees with a lack of out-of-plane distortions of the heme. For ferrous deoxy *Sp.so* Hb this can easily be understood in terms of the bis-histidine coordination. However, it is interesting to note that the heme plane is less distorted in case of the *S. solidissima* nHbCO than in the case of MbCO.

The spectra of nHbCO, recorded with laser powers of 1 and 17 mW, are shown in Figure A. It can be seen that even in the spectrum recorded with a laserpower of 1 mW a small fraction of photodissociation takes place (see also main text). Upon increase of the laser power to 17 mW, the intensity of the  $\nu_4$  marker line at 1460 cm<sup>-1</sup> increases, which indicates that the amount of ferrous species, due to photodissociation, increases. Note that the vinyl C<sub>a</sub>=C<sub>b</sub> stretching mode at 1626 cm<sup>-1</sup> in the 1 mW spectrum downshifts to 1619 cm<sup>-1</sup> in the 17 mW spectrum. A similar downshift of the C<sub>a</sub>=C<sub>b</sub> stretching mode upon CO photodissociation was observed for Mb, Cygb, and Ngb (3,4). The  $\nu_5$  marker line, which is situated around 1130 cm<sup>-1</sup>, doesn't shift upon CO photodissociation, which was also found for Ngb and Cygb (4). In contrast, for Mb, this line was found to downshift from 1135 cm<sup>-1</sup> to 1119 cm<sup>-1</sup> upon CO photodissociation (3).

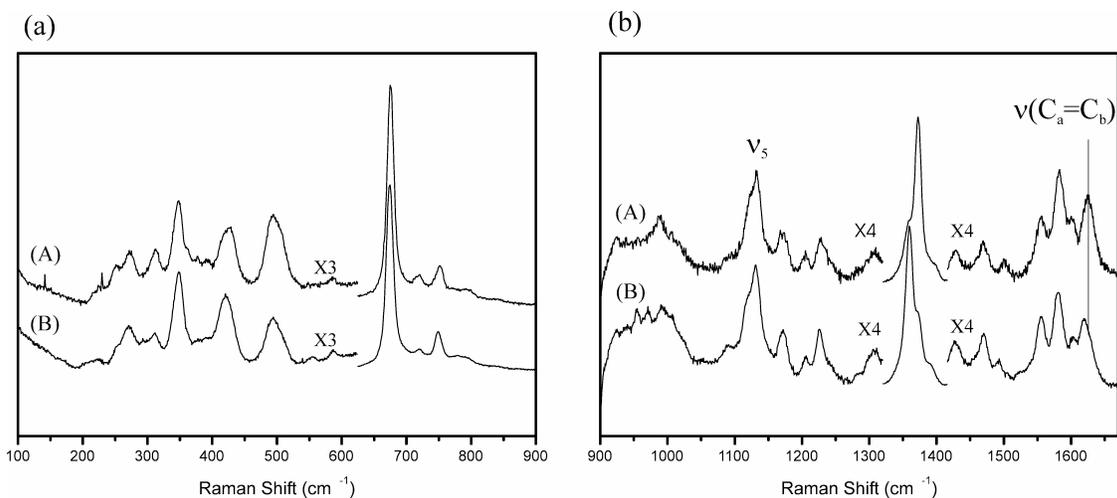


Figure A: (a) Low frequency and (b) high-frequency resonance Raman spectra of *S. solidissima* A) nHbCO (laser power 1 mW), B) nHbCO (laser power 17 mW).

In Figure B, the Fe-CO stretching modes of *S. solidissima* nHbCO are shown. From spectral deconvolution it can be seen that there are two CO coordination configurations: one with the Fe-CO stretching mode at  $494\text{ cm}^{-1}$  (75%) and one with this mode at  $513\text{ cm}^{-1}$  (25%) (dashed lines in Figure A).

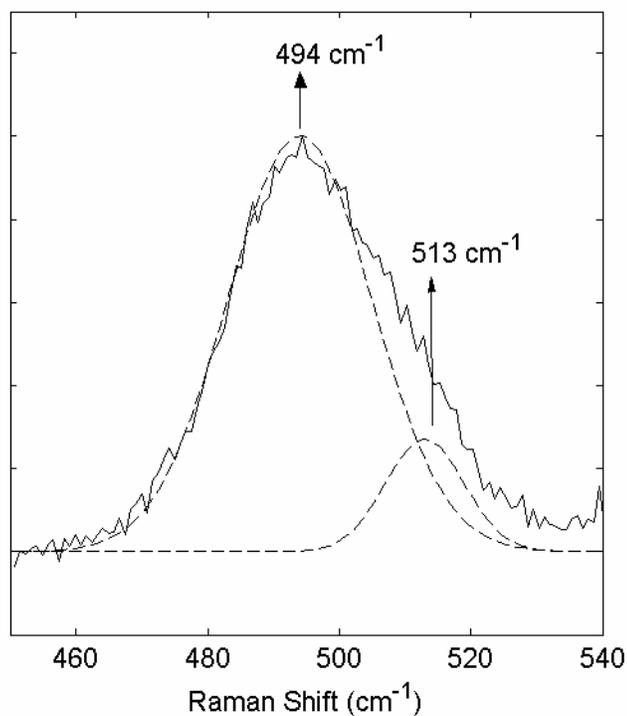


Figure B: Experimental Fe-CO stretching modes (solid lines). The dashed lines are the spectra obtained after spectral deconvolution.

## Reference List

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