The relaxed geometry for carbon monoxide ligated myoglobin was achieved with the aid of Gromacs version 5.0 and a modified gromos43a2 force field. Initial geometry was taken from x-ray structure of sperm whale myoglobin; protein data bank file 5jom. The structure was solvated with 6373 water molecules and relaxed using a steepest descent energy minimization protocol. The protein coordinates were restrained and the solvent was equilibrated for 10 ps in the canonical ensemble using a modified Berendsen thermostat and for 10 ps in the isothermal-isobaric ensemble incorporating the Parrinello-Rahman barostat. A series of structure files were then generated, displacing the iron and carbon monoxide atoms, as a rigid group, above and below the plane of the HEME.

In the relaxed protein the iron is below the plane of the HEME where Δr = -0.036 nm; towards the proximal histidine, HIS 93. The carbon of the carbon monoxide is 0.141 nm above the HEME. In the figure Δr = 0 nm is the point where the iron is in plane. Δr > 0 pushes the ligand closer to the distal histidine, HIS 64, and VAL 68; the region in red. To a lesser extent, it also approaches PHE 43 and ARG 45; the region in blue. The iron advances toward HIS 93, when Δr < 0, additionally causing a noticeable effect on HIS 97; the region in green.