Supporting Information for:

Docking and Migration of Carbon Monoxide in Nitrogenase – The Case for Gated Pockets from IR Spectroscopy and Molecular Dynamics

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Methods

All experimental data presented here were collected using a Bruker Vertex 70v FT-IR with the sample held at cryogenic temperatures inside a modified Oxford helium flow cryostat. Photolysis was performed using a Sutter Instruments xenon-arc lamp. All samples were purified, reacted with CO, and concentrated as previously reported.[1] Samples were held in custom built cells with Teflon spacers to give a pathlength of 70 microns.

We implemented the CHARMM27a[2] force field in the GROMACS[3] molecular dynamics package (version 4.5.3). The bonded parameters for the FeMo-cofactor were derived from nuclear resonant vibrational spectroscopy on the FeMo-cofactor[4] – however a 500 kj*mol-1*rad-2 potential was placed on the angular interaction between the μ^3-sulfurs, iron (vertex), and μ^2-sulfurs to prevent significant cofactor distortion. Equilibrium distances for the FeMo-cofactor were taken from the crystal structure (PDB: 3U7Q).[5] Van der Waals interactions for the cofactor were obtained from previous work on rubredoxin, using the nonbonding parameters for the bridging sulfurs and iron face in rubredoxin for the sulfurs and iron atoms respectively in FeMo-cofactor.[6] Likewise, the iron nonbonding parameters were used for molybdenum the CHarges from Electrostatic Potentials using a Grid (CHELPG)[7] scheme as implemented in GAUSSIAN 09 (Rev A.02),[8] in which atomic charges

are fitted to reproduce the molecular electrostatic potential (ESP) at a number of points around the molecule. For Fe and Mo atoms, Breneman radii of 1.3 Å were used,[9] and the default implemented radii for the rest of the atoms. The CHELPG scheme was applied to our density functional theory (DFT) FeMo-cofactor model with CO terminally bound at Fe2.[10] For the DFT modeling, the PBE[11] functional and LACV3P** basis set as implemented in JAGUAR 7.9[12] were used. The LACV3P** basis set implies Los Alamos effective core potential for Fe and Mo, and 6-311G**[13, 14] for all other atoms. We found[15] this method has performed well for the FeMo-cofactor bound CO frequencies. The cofactor polarization effect induced by the protein environment was accounted for via the self-consistent reaction field (SCRF) procedure with $\varepsilon = 4$ and the rest of the parameters as default for water. Waters used in the molecular dynamics simulations were modeled explicitly by the TIP3P three site model.[16] Homocitrate was parameterized from the CGenFF 2b7 force field.[17] Charges for CO were obtained from previous work on free CO dipole calculations.[18, 19]

Initial coordinates were derived from a previous 1.0 Å resolution X-ray structure (PDB ID: 3U7Q).[5] The system was truncated to the α1 subunit and all non-proteic and non-cofactor atoms were removed. A single carbon monoxide was added proximal to the Fe2 of FeMo-cofactor (1.9 Å Fe2-C as the initial distance). The protein was inserted into a rectangular box with a minimum distance of 15 Å between the protein and any boundary giving the dimensions 107.5x99.6x82.5 Å along each axis. The system was solvated with 26,769 water molecules. Towards modeling a system of biological relevance, sodium and chlorine ions were added to neutralize the system and bring the ionic strength of the system to 0.15M.

The entire system was energy minimized using a double precision version of GROMACS to a maximum interatomic force below 10 kJ*mol-1*nm-1. Classical dynamics were then held at multiple temperatures using a velocity rescaling thermostat[20] with a time constant of 0.1 ps. The pressure of the system was held at 1 atm using a Berendsen[21] barostat with a time coupling constant of 0.5ps. The total duration of the simulation was 10ns with a time step of 0.2 femtoseconds and saving trajectory snapshots every 100 femtoseconds.

Trajectories were analyzed by determining the minimum distance between CO and the protein residues to identify channel residues (2.7 angstrom cutoff). Moreover, distance as a function of simulation time was determined between the center of masses for CO and the Fe2 of FeMo-cofactor using the g_dist utility freely available in Gromacs.

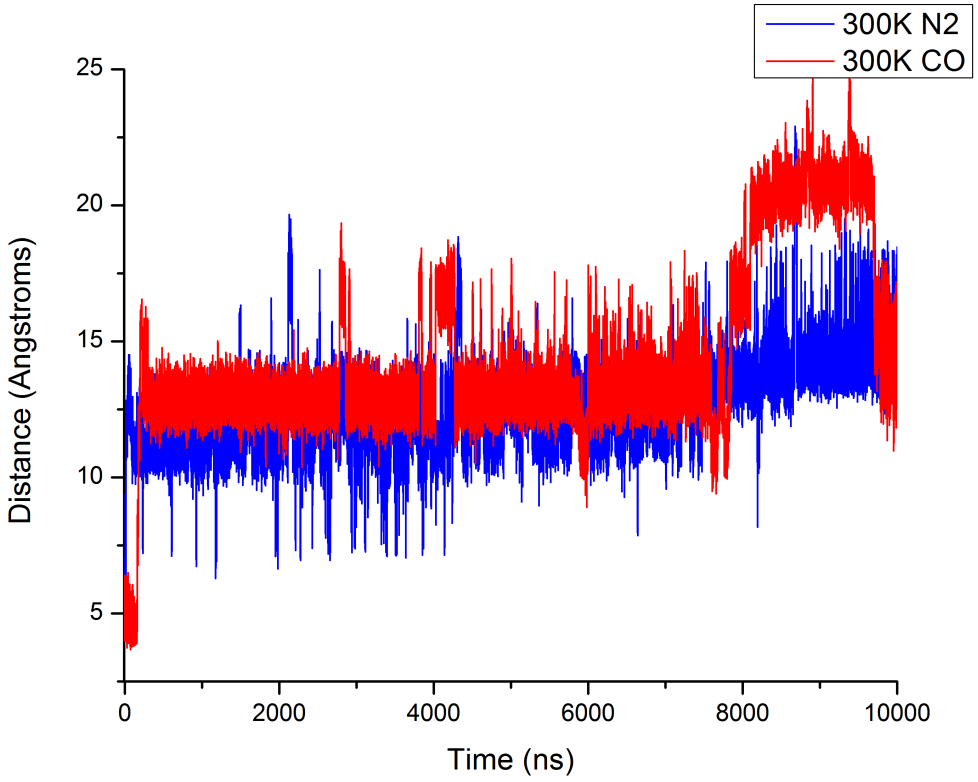

Figure S1: A dinitrogen simulation was performed at 300K and the gas followed a similar path to the simulations conducted with CO. The bonded force constant was derived from the Dunham coefficient for N_2.

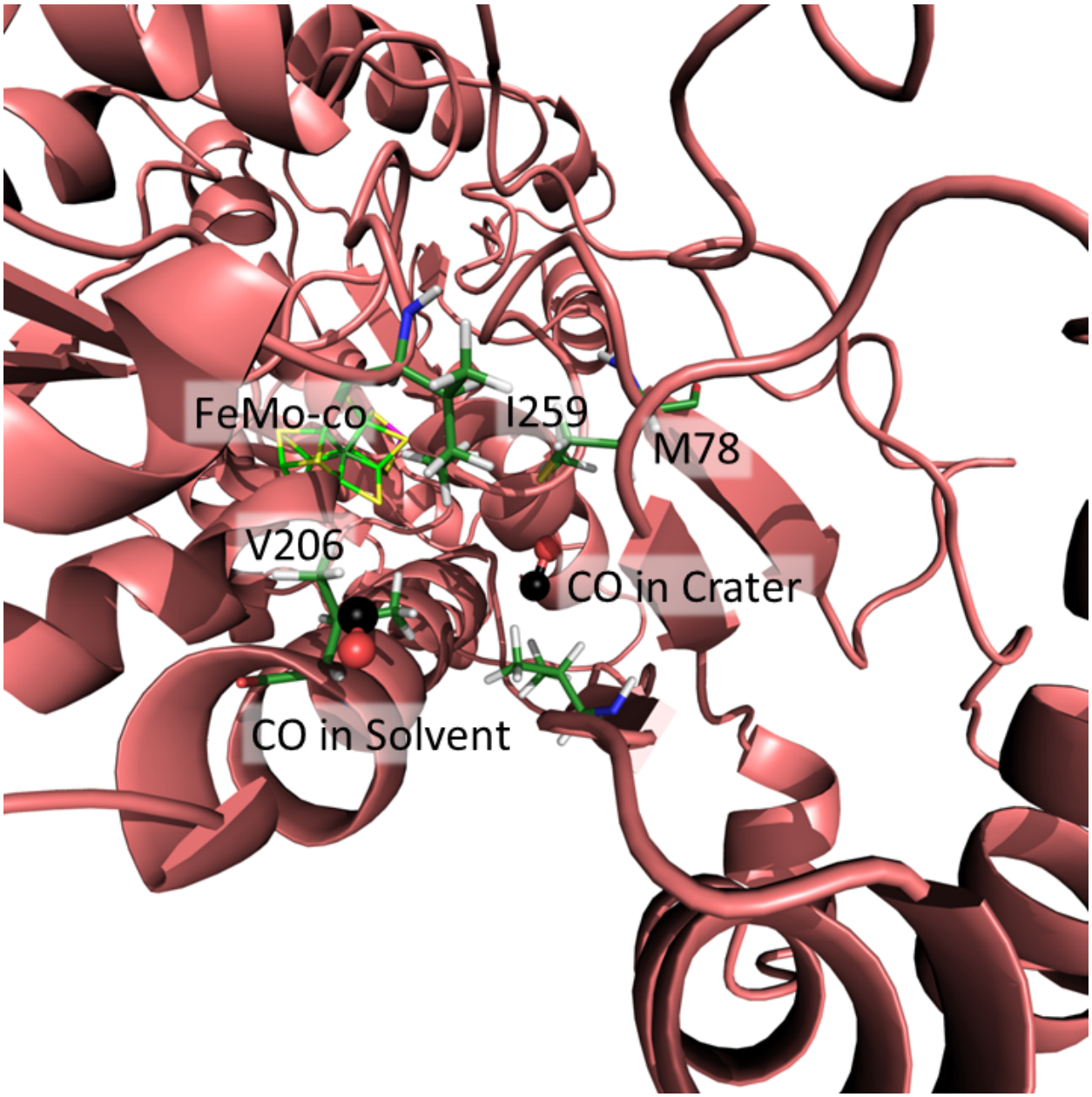

Figure S2: The "crater region" of the proposed channel where CO is initially in contact with the solvent and the super imposed position of CO in the solvent space outside of the crater.

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