Supplementary Information for

Mechanism of the Nitric Oxide Dioxygenase Reaction of *Mycobacterium tuberculosis* Truncated Hemoglobin N

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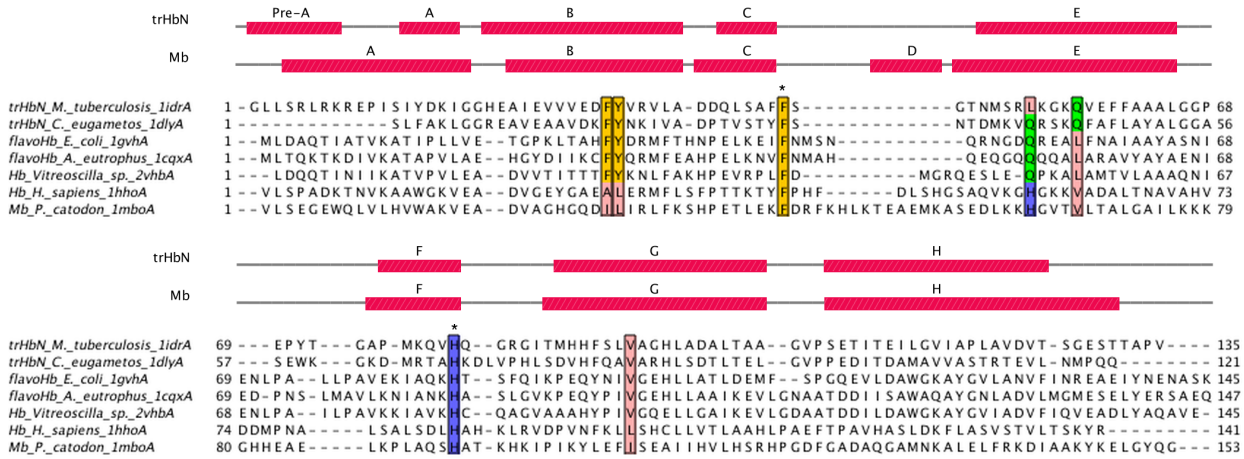

Figure S1. Multiple sequence alignment of selected globins: truncated hemoglobins N (trHbN) from *Mycobacterium tuberculosis* and unicellular alga *Chlamydomonas eugametos*, bacterial flavohemoglobins (flavoHb) from *Escherichia coli* and *Alcaligenes eutrophus*, bacterial single-domain hemoglobin (Hb) from *Vitreoscilla sp.*, and mammalian hemoglobin (Hb) and myoglobin (Mb) from *Homo sapiens* and *Physeter catodon*. trHbNs nomenclature follows the convention established for *P. catodon* Mb: each helix is designated by a letter (A through H) and each residue is numbered based on its position in that helix. The helical regions of *M. tuberculosis* trHbN and *P. catodon* Mb are represented by red bars. Of note for trHbN are the truncations in the A helix sequence, the presence of the pre-A helical motif and the absence of a D helix. Key conserved residues are highlighted in color and framed. The proximal HisF8 and distal PheCD1 residues, conserved in all globins, are marked with an asterisk. The polar TyrB10 and aromatic PheB9 residues (highlighted in yellow) are highly conserved among trHbs and flavoHbs but are substituted by aliphatic amino acids in Mb. The polar GlnE11 residue (highlighted in green) is conserved in trHbs but is substituted by Leu in flavoHbs and bacterial Hbs, and by Val in mammalian globins. Inversely, the classical HisE7 position in mammalian globins (in blue) is substituted by Gln (in green) in flavoHbs and bacterial Hbs and by Leu (in pink) in trHbN of *M. tuberculosis*. Alignments were obtained using PROMALS3D Web server [Pei, J.; Grishin, N. V. *Methods Mol. Biol*. **2013**, *1097*, 263-271]. and manually adjusted for optimal matching of critical residues using Jalview 2 multiple sequence alignment editor and analysis workbench [Waterhouse A. M.; Procter J. B.; Martin D. M.; Clamp M.; Barton G. J. *Bioinformatics* **2009**, *25*, 1189–1191]. Secondary structure annotations were generated using Jalview 2.

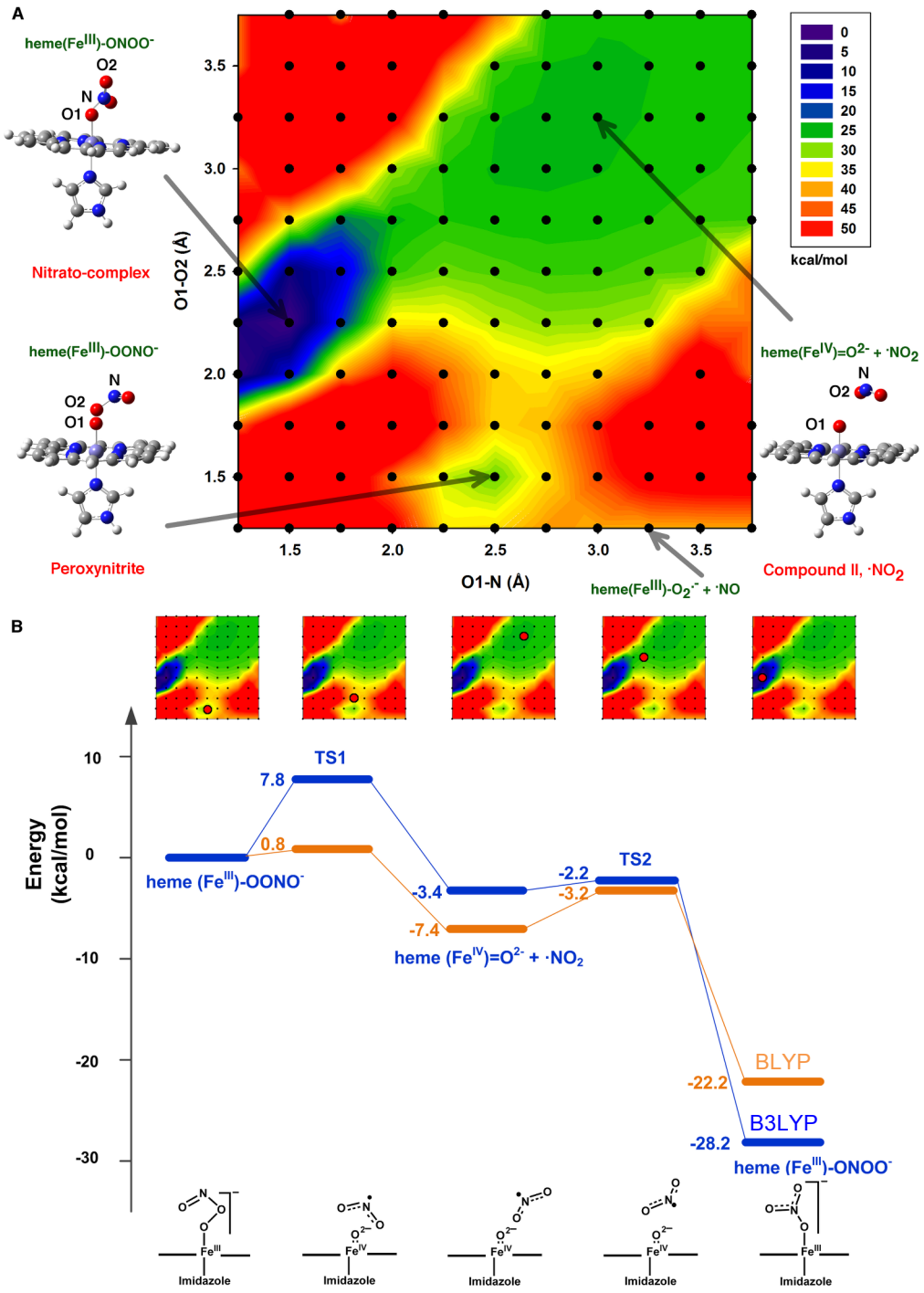

Figure S2. Panel (A) 2D potential energy surface (PES) of the NOD isomerization reaction in gas phase. Each black dot corresponds to a grid point where an individual geometry optimization with fixed O1-O2 and O1-N distances was performed at the UB3LYP/6-311G(d,p) level of theory (using ECP/LANL2DZ for Fe). The wave function of the peroxynitrite complex is constructed as a spin doublet using the wave functions of fragments as initial guess (iron(II)-porphine fragment and O_2-ligand fragment both in triplet states and nitric-oxide fragment in doublet state). The reaction coordinates were fixed at values between 1.25 and 3.75 Å and scanned in 0.25 Å increments. The O1-O2 distance describes the cleavage of the O-O peroxo bond of the heme-bound peroxynitrite complex, and the O1-N distance describes the formation of the O-N bond of the bound nitrate complex. Optimized structures of the gas-phase model are presented for three positions along the reaction pathway (indicated by black arrows). The potential energies are defined relative to that of the bound product, the minimum-energy structure on the 2D PES. Panel (B) Potential energies at grid points closest to each actual state (i.e. intermediates, transition states and bound product) of the NOD isomerization reaction relative to that of peroxynitrite, calculated with B3LYP (blue) and BLYP (orange) density functionals. The position of each of the five energy levels on the 2D PES is indicated above the graph (red dots), and a schematic of each complex is shown below.

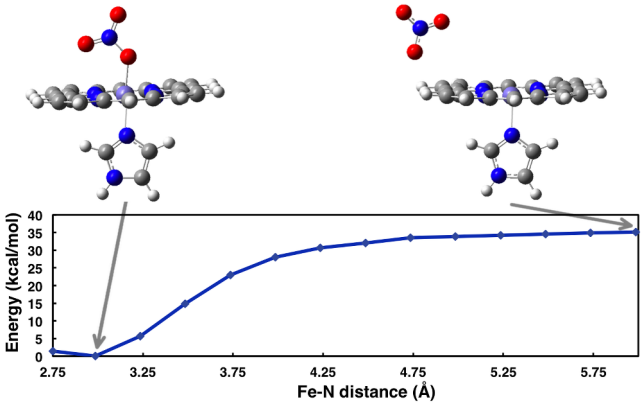

Figure S3. PES of the NOD product release in gas phase calculated from geometry optimizations with Fe-N distance scanned from 2.75 to 6.00 Å, in 0.25 Å increments. Each point on the potential energy profile corresponds to an individual geometry optimization at the UB3LYP/6-311G(d,p) level of theory with ECP/LANL2DZ for Fe. The minimum energy corresponds to bound NO_3^- at Fe-N distance of 3.00 Å. NO_3^- is fully dissociated at Fe-N distances larger than 4.75 Å. The energetic difference between bound and dissociated NO_3^- is 35 kcal/mol. Optimized structures of bound and fully dissociated NO_3^- are shown above the PES (indicated by black arrows).

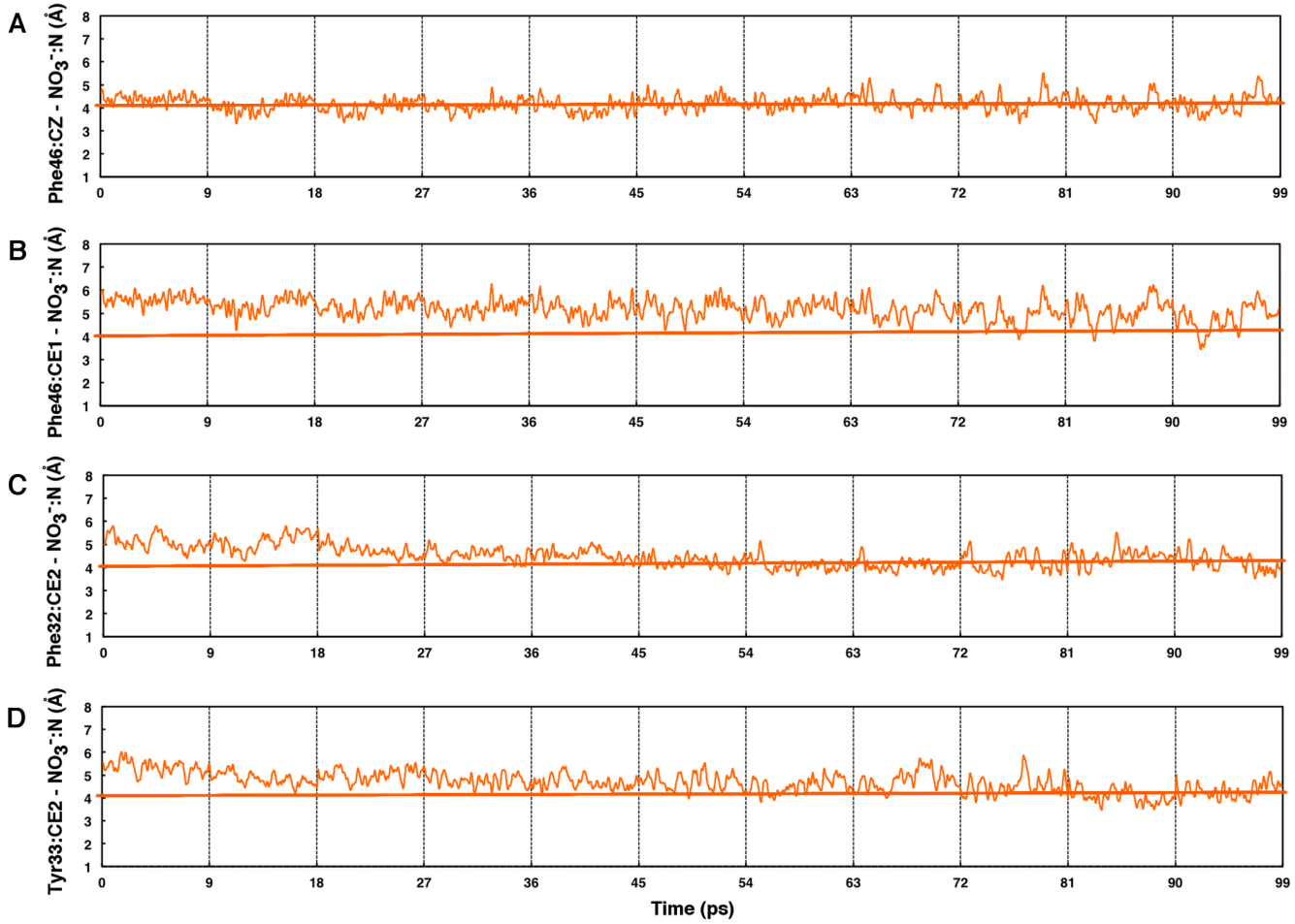

Figure S4. trHbN dynamics during the NOD product release. Each 9-ps segment (delimited by vertical dashed lines) corresponds to one of the 14 red points on the 1D PMF of Figure 5, in order of increasing Fe-N distance. The time series represent the evolution of the distances between carbon atoms of the aromatic rings of Phe46(CD1) (panels A and B), Phe32(B9) (panel C), and Tyr33(B10) (panel D) and the nitrogen atom of the NO_3^- anion. Phe46(CD1), Phe32(B9), and Tyr33(B10) favorably interact with NO_3^- via C−H···O hydrogen bonds. Stabilizing C−H···O contacts, formed when C−N distances are less than 4 Å, are indicated with continuous horizontal lines in panels A-D.