Supplementary Information for

***Mycobacterium tuberculosis* DosS binds H_2S through its Fe^{3+} heme iron to regulate the Dos dormancy regulon**

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Movies S1-S3

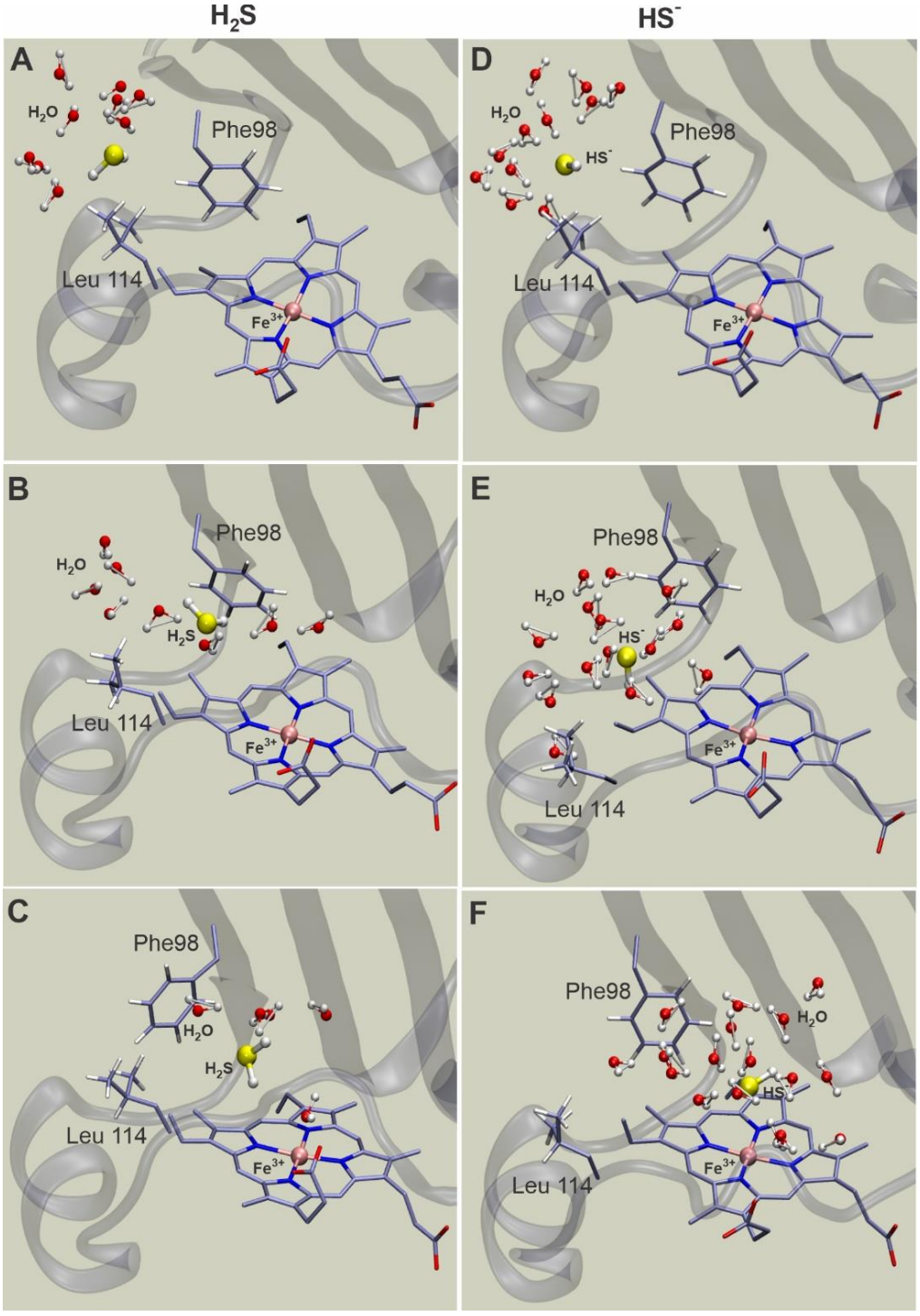

Fig. S1. Steered Molecular Dynamics (sMD) modeling of sulfide entry into the DosS heme pocket. Typical snapshots of H_2S (A-C), and HS^- (D-F) along the entry path into the DosS heme pocket, representing sulfide ligands at a distance of 11, 8 and 5 Å (upper, medium, and lower panels, respectively) from the heme iron.

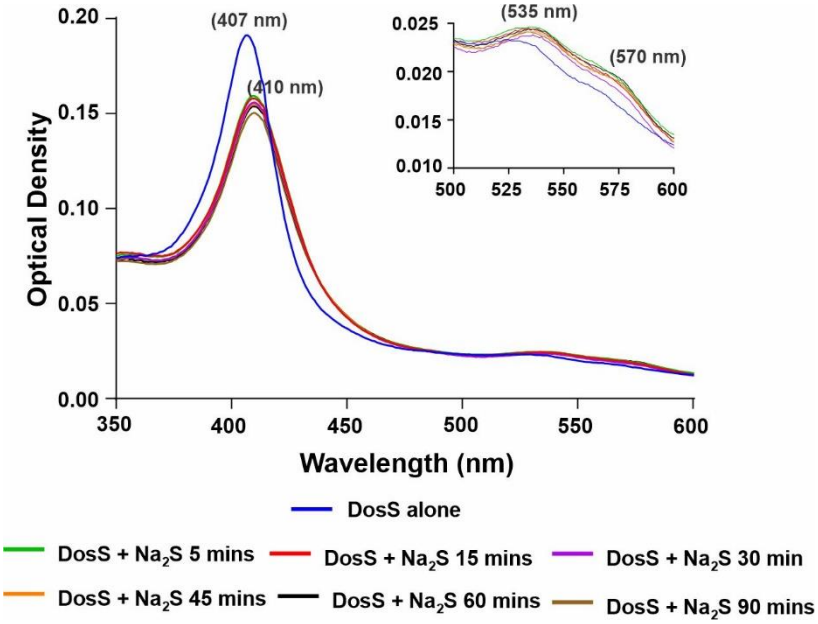

Fig. S2. UV-Vis time-course scan of DosS and Na_2S: Representative UV-Visible spectra of the Fe^{3+} form of DosS (3 µM) for 5-90 minutes following the addition of 30 µM Na_2S. (*Inset*) Absorption spectra replotted to highlight the α (570 nm) and β (535 nm) peaks.

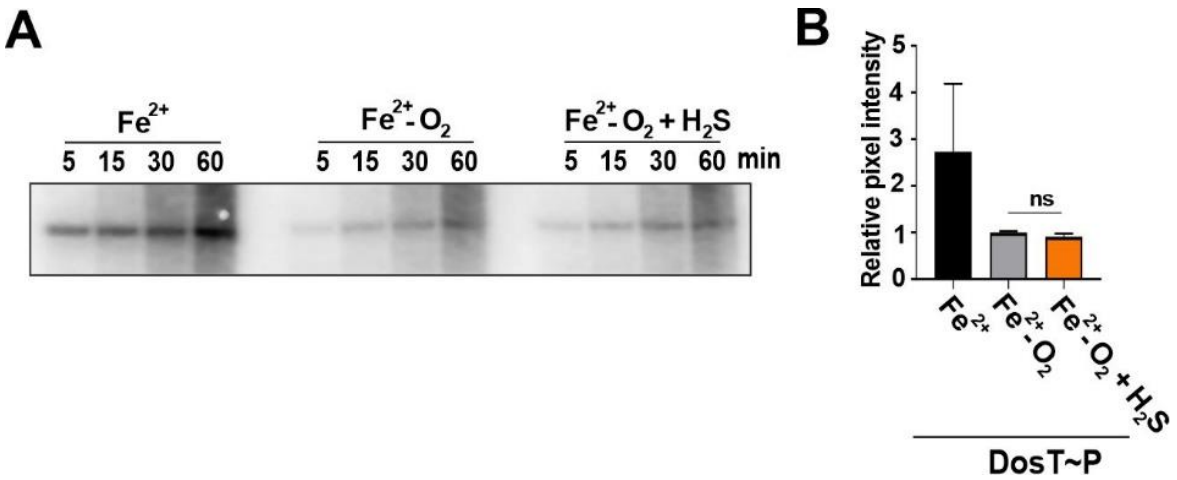

Fig. S3. Autophosphorylation assay. (A) Representative autoradiogram of PAGE-resolved γ-32P-labeled recombinant DosT following autophosphorylation in the presence of γ-32P-ATP alone (left and center panels) or with γ-32P-ATP in the presence of 100 µM Na2S (right panel). (B) Densitometric quantitation of DosS bands in (A) at 60 min (n=3).

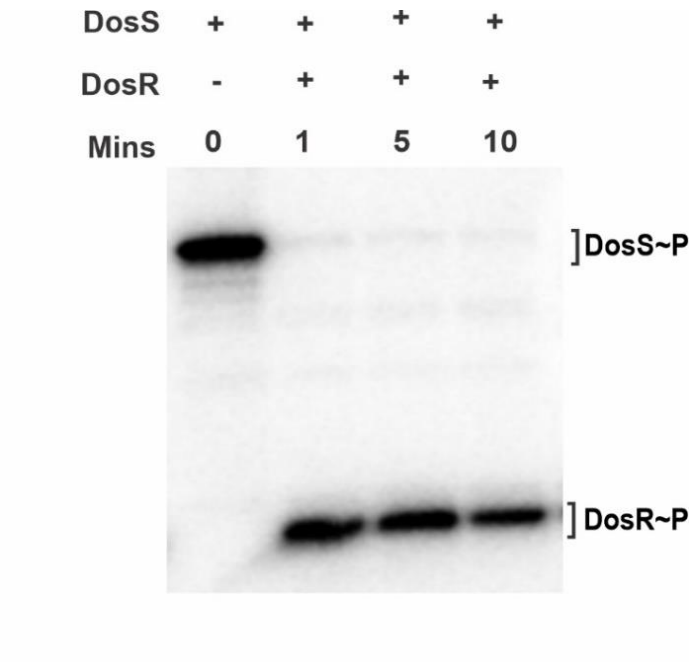

Fig. S4. Transphosphorylation assay. Autoradiogram of PAGE-resolved ^{32}P-labeled DosS in the Fe^{3+} form alone after 60 min of autophosphorylation (left lane), and following addition of recombinant DosR to the autokinase reaction. The reactions were stopped 1-10 min following addition of DosR.

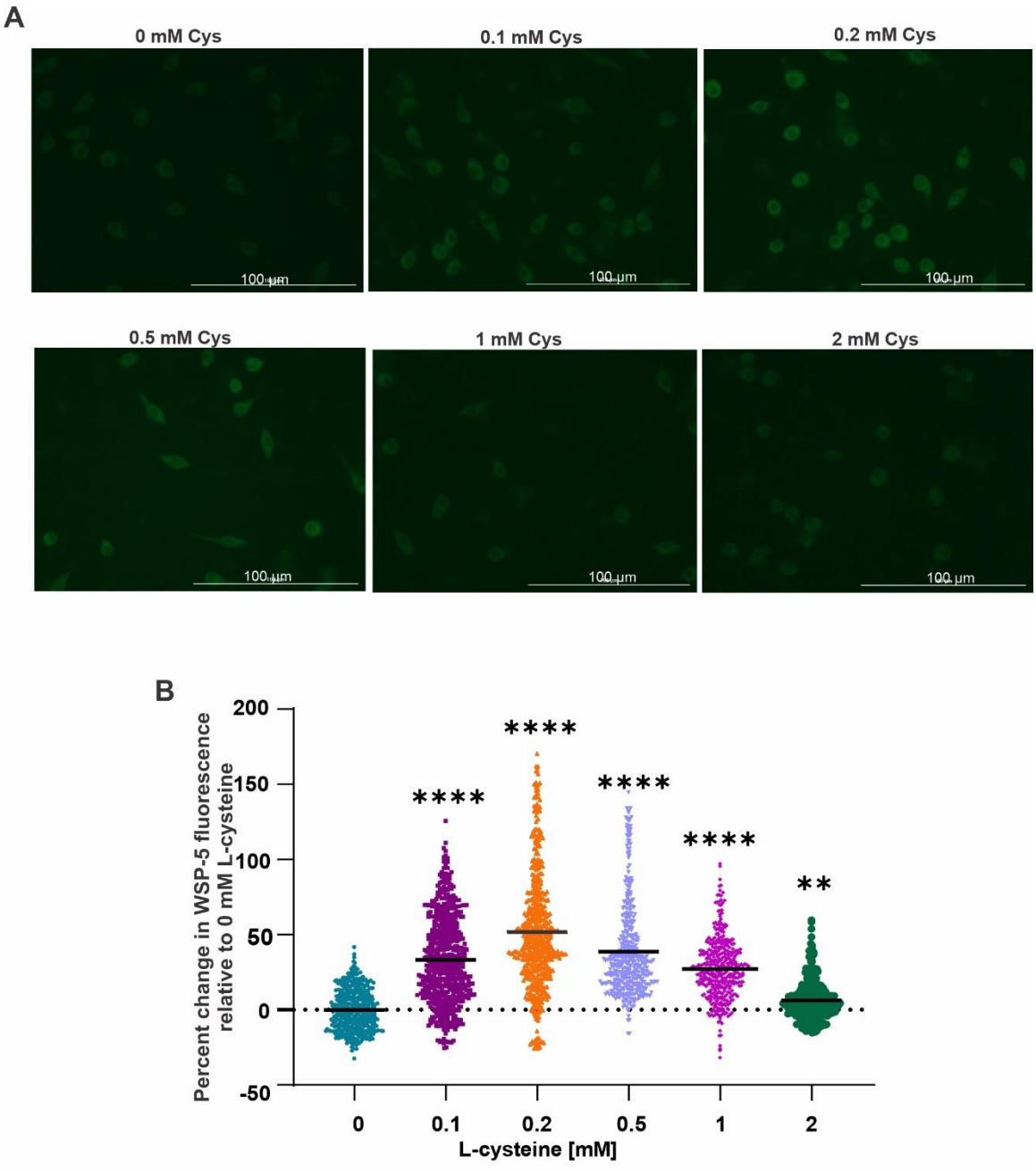

Fig. S5. **Detection of intracellular H_2S.** (A) Representative images of intracellular WSP-5 fluorescence in RAW 264.7 macrophages grown in media containing 0-2.0 mM L-cysteine (Cys). (B) Percent change in intracellular H_2S-dependent probe fluorescence levels in RAW 264.7 macrophages in presence of 0-2.0 mM L-cysteine as determined by WSP-5 fluorescence measurements in individual cells. The data were analyzed using Kruskal-Wallis test with Holm-Sidak multiple comparisons. The solid black line represents the mean value of the group. **P < 0.01 and ****P < 0.0001 vs 0 mM L-cysteine control.

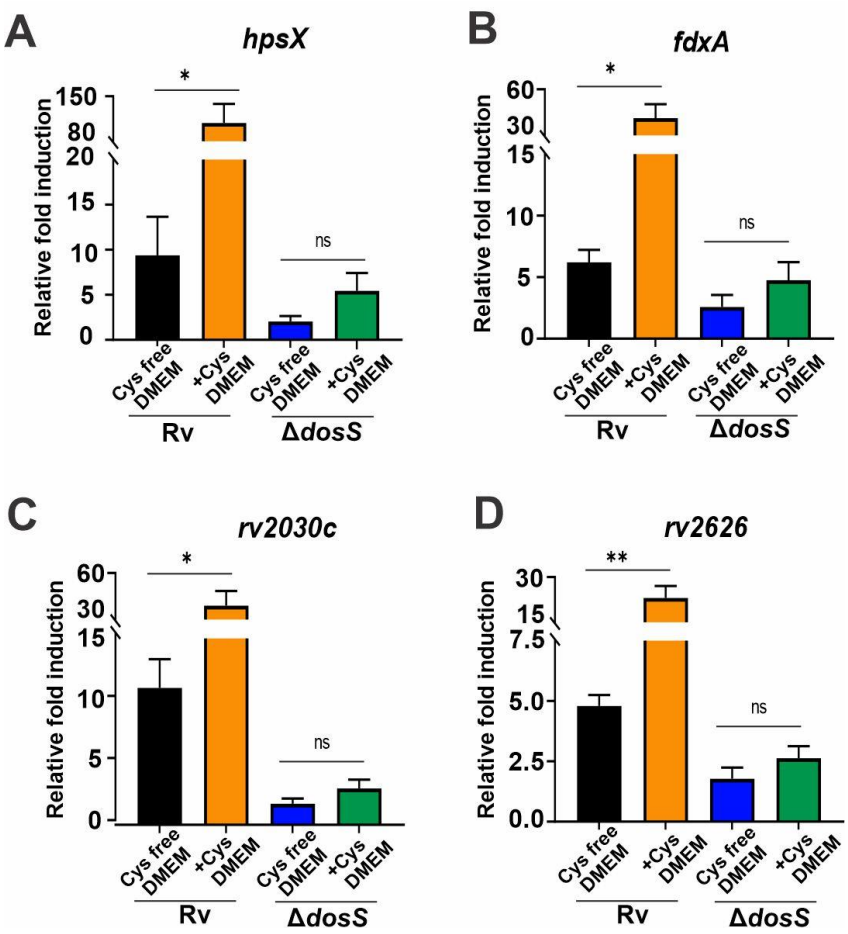

Fig. S6. Effect of Cysteine depletion in DMEM culture on expression of DosR regulon genes. (A-D) Expression of representative Dos regulon genes in WT and ΔdosS *Mtb* isolated from infected RAW 264.7 macrophages grown cysteine-free DMEM or DMEM containing 200 µM L-Cys for 24 hours (n=3). RT-qPCR gene expression data from macrophage-isolated *Mtb* are shown relative to gene expression in WT and ΔdosS *Mtb* cultures exposed to DMEM. Data are shown as the mean ± SEM and were analyzed using a one-way ANOVA with Tukey post-hoc test performed using GraphPad Prism version 9. *p< 0.05 and **P < 0.01.

Table S1: Comparison of Biophysical Parameters of Sulfide-Binding Proteins

Protein	**UV-vis** (nm)	**EPR** (g-values)	**K_D** (μM)	**Reference**
Mtb DosS	415, 535, 570 (s)	2.67, 2.26, 1.76	5.64 (H_2S)	This work
L. pectinata Hb I	425, 545, 573 (s)	2.67, 2.24, 1.84	0.09 (XS_T)	(1)
Human Hemoglobin	423, 577, 541	2.51, 2.25, 1.86	17 (XS_T)	(2)
Bovine Hemoglobin	425, 542, 575(s)	2.55, 2.26, 1.88	7(XS_T)	(3)
Equine Myoglobin	427, 547, 580	2.56, 2.25, 1.83	96 (XS_T)	(4), (5)
Human Myeloperoxidase	432, 625	2.567, 2.274, 1.850; 2.512, 2.262, 1.875	< 12 (XS_T)	(6)
Microperoxidase	414, 536, 568	--	219 (calc) (XS_T)	(7)
Truncated *B. subtilis* Hb	427, 550, 577	--	0.2 (XS_T)	(8)
Truncated *T. fusca* Hb	425, 550, 575	--	0.36 (XS_T)	(8)
E. coli DOS (heme-regulated TCS PDE)	427,546, 579	--	ca. 200 (XS_T) (based on PDE activation)	(9)
*Anaeromyxobacter Af*GcHK (heme-regulated TCS His kinase)	423-426,549-551	--	200-5000 (XS_T) for binding, although no autokinase activation with 10 mM	(10)

Note: Hemeproteins that get reduced on sulfide binding do not show the EPR spectrum and hence were not used for comparison eg., cytochrome c oxidase, neuroglobin, cytochrome c.
H_2S = based on H_2S concentration
XS_T = based on concentration of total sulfide species ($H_2S + HS^- + S^{2-}$)
TCS = two-component system
(s) = shoulder
calc = calculated

Table S2. Comparison of structural and electronic parameters of H_2S and HS^- bound states obtained from QM/MM calculations

Parameter	H_2S	HS^-
dFe-S	2.35 Å	2.17 Å
H_2S or HS^- Mulliken population	0.5543	0.0984
dFe-NHis	2.06 Å	2.09 Å
dS-H1 dS-H2	towards Tyr 1.36 Å towards water1.39 Å	1.35 Å
H1 Mulliken population H2 Mulliken population	towards Tyr 0.2242 towards water 0.2644	S-H 0.1698 Tyr-H 0.4458

Table S3. DNA Primers used for qRT-PCR.

Primer	Primer Sequence (5’ to 3’)
SigA-For	TCGGTTCGCGCCTACCT
SigA-Rev	TGGCTAGCTCGACCTCTTCCT
hspX-For	CGCACCGAGCAGAAGGAC
hsp-Rev	CCGCCACCGACACAGTAA
fdxA-For	CCTATGTGATCGGTAGTGA
fdxA-Rev	GGGTTGATGTAGAGCATT
rv2030-For	GAATAGTGGTGTGGGCTCATAA
rv2030-Rev	CGTATCGCTCACGGACTATCT
rv2626-For	CGACCGCGACATTGTGAT
rv2626-Rev	CATCGACGTAGTAGATGCTGT

movie_sh2_lado.mp4

Movie S1. Steered Molecular Dynamics modeling of H_2S entry into the DosS heme pocket. The trajectory of H_2S [shown in yellow (S) and white (H) spheres] approaching the DosS heme is shown. H_2S first approaches the Y97-F98 pair, and then passes between F98 and the L114-P115 pair to enter the heme pocket, where it interacts with distal Y171. The H_2S solvation shell waters are represented as small red and white triangles. The overall DosS secondary structure is shown as a cyan tube. Red = oxygen, Blue = nitrogen, Pink = heme center. Movie represents 2.8 ns of simulation with a ligand pulling speed of 0.0025 Â/ps.

movie_sh_lado.mp4

Movie S2. Steered Molecular Dynamics modeling of HS^- entry into the DosS heme pocket. The trajectory of HS^- [shown in yellow (S) and white (H) spheres] approaching the DosS heme is shown. HS^- first approaches the Y97-F98 pair. The barrier for HS^- entry is formed by disrupting the F98-L114 interaction, which is clearly seen when the L114 sidechain changes separates from F98. HS^- can then pass between F98 and the L114-P115 pair to enter the heme pocket, where it interacts with distal Y171. The larger barrier for HS^- is due to the fact that while HS^- enters almost dry, the negatively charged ligand is always solvated and accompanied by several water molecules. The HS^- solvation shell waters are represented as small red and white triangles. The overall DosS secondary structure is shown as a cyan tube. Red = oxygen, Blue = nitrogen, Pink = heme center. Movie represents 2.8 ns of simulation with a ligand pulling speed of 0.0025 Â/ps.

Video Protonation of H2S - Short.mp4

Movie S3. QM/MM modeling of deprotonation of H_2S in DosS heme pocket. Deprotonation of H_2S [shown in yellow (S) and white (H) spheres] following binding to the DosS Fe^{3+} heme iron is shown. The proton transfer to a nearby heme propionate group via a water bridge is spontaneous and occurs within 0.35 ps. The QM subsystem is depicted in balls and sticks. The overall DosS secondary structure is shown as a gray ribbon. Red = oxygen, Blue = nitrogen, Pink = heme center. Movie represents 0.4 ns of simulation.

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