

**Molecular dynamics of deoxyhemoglobin complexes
with the bis(μ -thiosulfato-*S*)bis(dinitrosylferrate)(2–) anion**

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Calculation methods

The iron atoms of NIC **1** were considered in the electronic structure calculations in the high-spin $3d^7$ state with anti-parallel orientations of spins on two iron centers (e.g., the previous work¹²). The spins on NO groups are anti-ferromagnetically bound with the spins of coordinating Fe⁺ in [Fe(NO)₂]⁺. In the end that makes the total spin of the ligand equal 0. Amber atomic charges^{S1} for globin and heme were used for docking. For NIC **1** we have calculated the HF/6-31G(d) RESP charges (Figure 1s). In docking the ligand binding sites were searched in the rectangular box with a 10 Å distances between the protein and the box borders. The first 100 obtained ligand orientations were considered. Molecular structures and topologies were prepared using AmberTools.^{S2} The AFF19SB potentials were used for Hb, GAFF2 for NIC **1**, and TIP3P for water. The missing intramolecular parameters of the ligand were determined from the B3LYP/6-31G+(d,p) calculations of the mononuclear complex [Fe(NO)₂(SSO₃)₂]³⁻.

In the MD simulations the protein – ligand complex was placed in the rectangular box 92 Å x 86 Å x 83 Å, i.e. with ~10 Å distances between the protein and the box borders, filled with the TIP3P water molecules. The total charge negative charge –8 of the system was neutralized by adding Na⁺ ions. The MD calculations were performed for periodic boundary system at the temperature T=300K and the pressure of 1 atm using the Langevine thermostat, the Nose-Hoover barostat and default other parameters. The distance between the heme iron and the proximal Histidine residue was maintained by adding the corresponding extra bond in topology files. The system was minimized for 100 steps. The stepwise heating to 300 K and equilibration was performed for 25 ps of MD. After that the 50-ns MD phase was considered. The relative ligand binding affinities at the found sites s1 and s2 were assessed using MM-GBSA method based on the productive trajectories from the last 10-ns of MD of stable positions.

The SMD simulations of dissociation of the protein – ligand complexes in the s1 and s6 sites were performed via applying the force to the ligand in the direction to the border of the periodic cell that corresponds to the shortest dissociation path. The applied potential had the form $U(\vec{R}, t) = \frac{1}{2}k(vt - (\vec{R}(t) - \vec{R}_0) \cdot \vec{n})^2$. In this equation, $\vec{R}$ – is the time-dependent radius-vector of the center of mass of DNIC **1** that is equal $N_s \cdot \Delta t$. N_s is the number of modelling steps for time period t with the time step $\Delta t = 1$ fs, $\vec{n}$ – is unit vector in the direction of the applied force. The pulling velocity v was $8 \cdot 10^{-5}$ Å⁻¹ fs, the harmonic force constant k was 10 kcal mol⁻¹ Å⁻². The ligand pulling was performed until full dissociation of the protein – ligand complex.

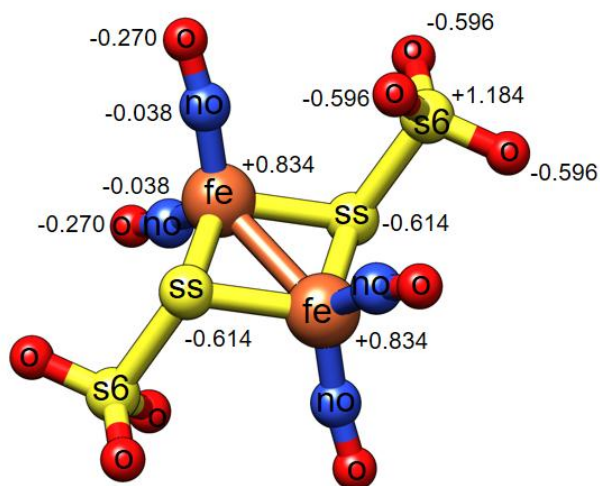


Figure S1 The structure of NIC 1 with the GAFF2 atom types and the HF/6-31G(d) RESP partial charges.

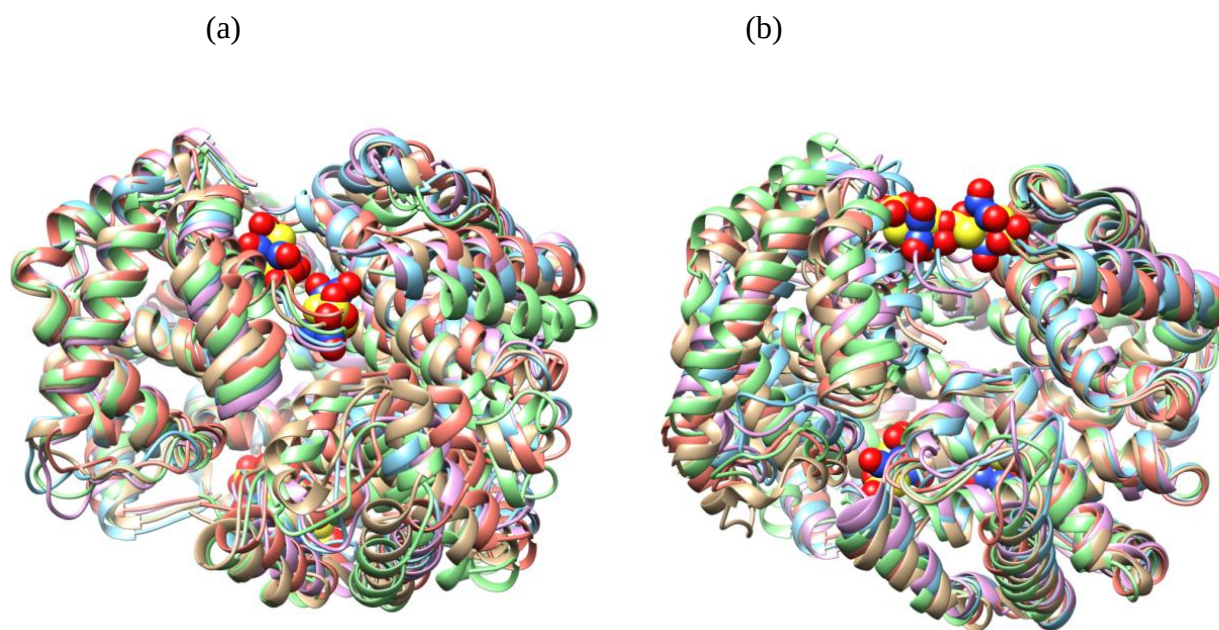


Figure S2 The ligand positions on Hb in the s1 (a) and s2 (b) sites in the end of the 50-ns MD trajectories. Superpositions of the Hb structures from four trajectories for each of the bound ligand cases are shown.

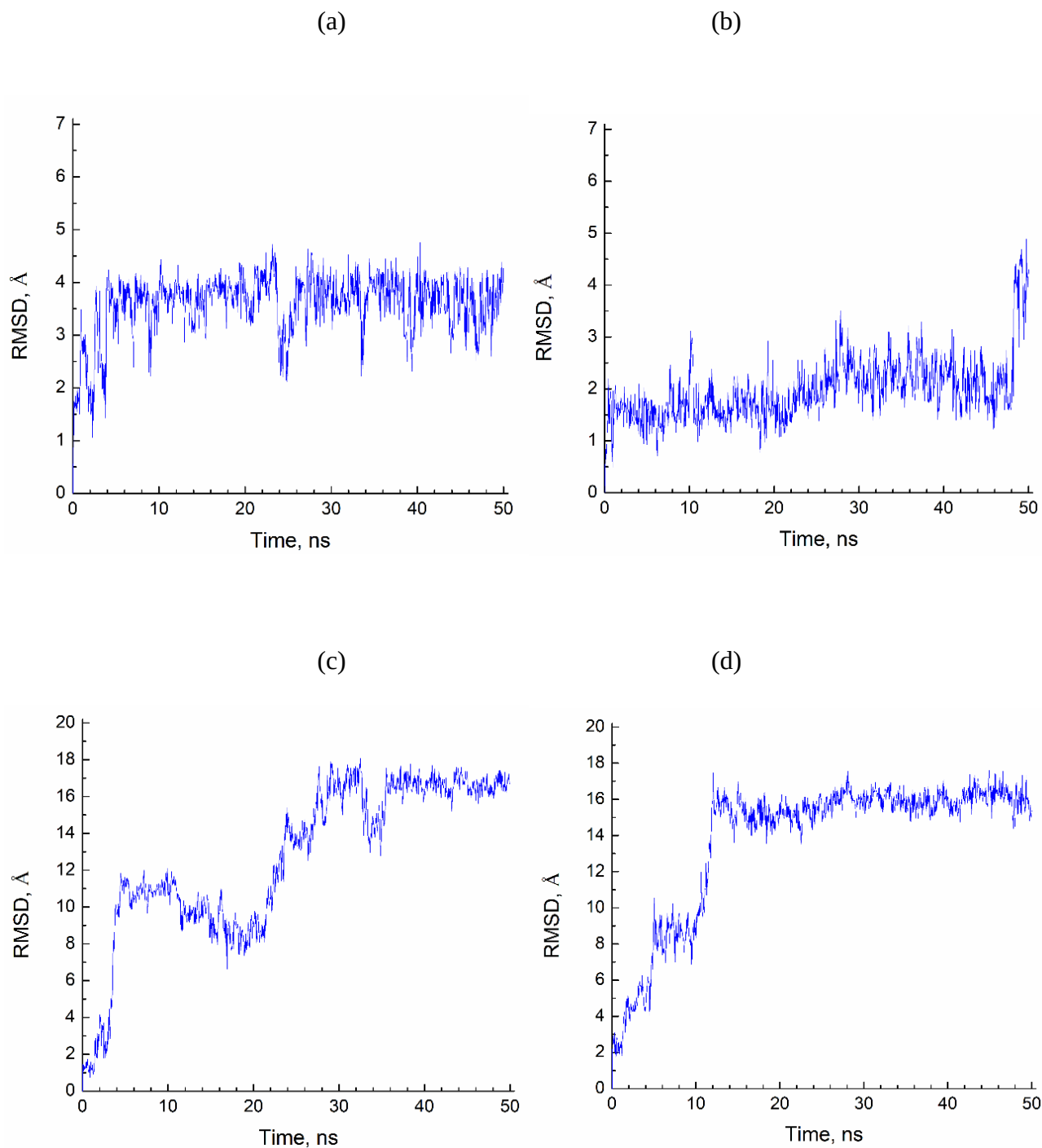


Figure S3 The RMSDs of NIC **1** relative to the main chain of Hb along the calculated MD trajectories for the s1 poses p1 (a) and p2 (b), and s2 poses p1 (c) and p2 (d) cases.

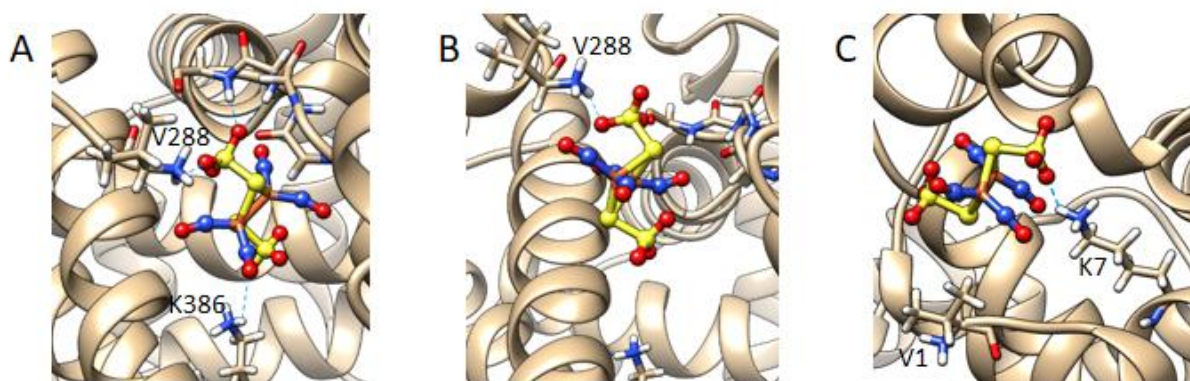


Figure S4 The ligand positions at the maxima on the force profiles on Fig. 3 for the s1 site

References

- S1 AMBER parameter database, <http://amber.manchester.ac.uk>.
- S2 D. A. Case, H. M. Aktulga, K. Belfon, D. S. Cerutti, G. A. Cisneros, V. W. D. Cruzeiro, N. Forouzes, T. J. Giese, A. W. Götz, H. Gohlke, S. Izadi, K. Kasavajhala, M. C. Kaymak, E. King, T. Kurtzman, T.-S. Lee, P. Li, J. Liu, T. Luchko, R. Luo, M. Manathunga, M. R. Machado, H. M. Nguyen, K. A. O'Hearn, A. V. Onufriev, F. Pan, S. Pantano, R. Qi, A. Rahnamoun, A. Risheh, S. Schott-Verdugo, A. Shajan, J. Swails, J. Wang, H. Wei, X. Wu, Y. Wu, S. Zhang, S. Zhao, Q. Zhu, T. E. Cheatham III, D. R. Roe, A. Roitberg, C. Simmerling, D. M. York, M. C. Nagan and K. M. Merz Jr, *J. Chem. Inf. Model.*, 2023, **63**, 6183. <https://doi.org/10.1021/acs.jcim.3c01153>.