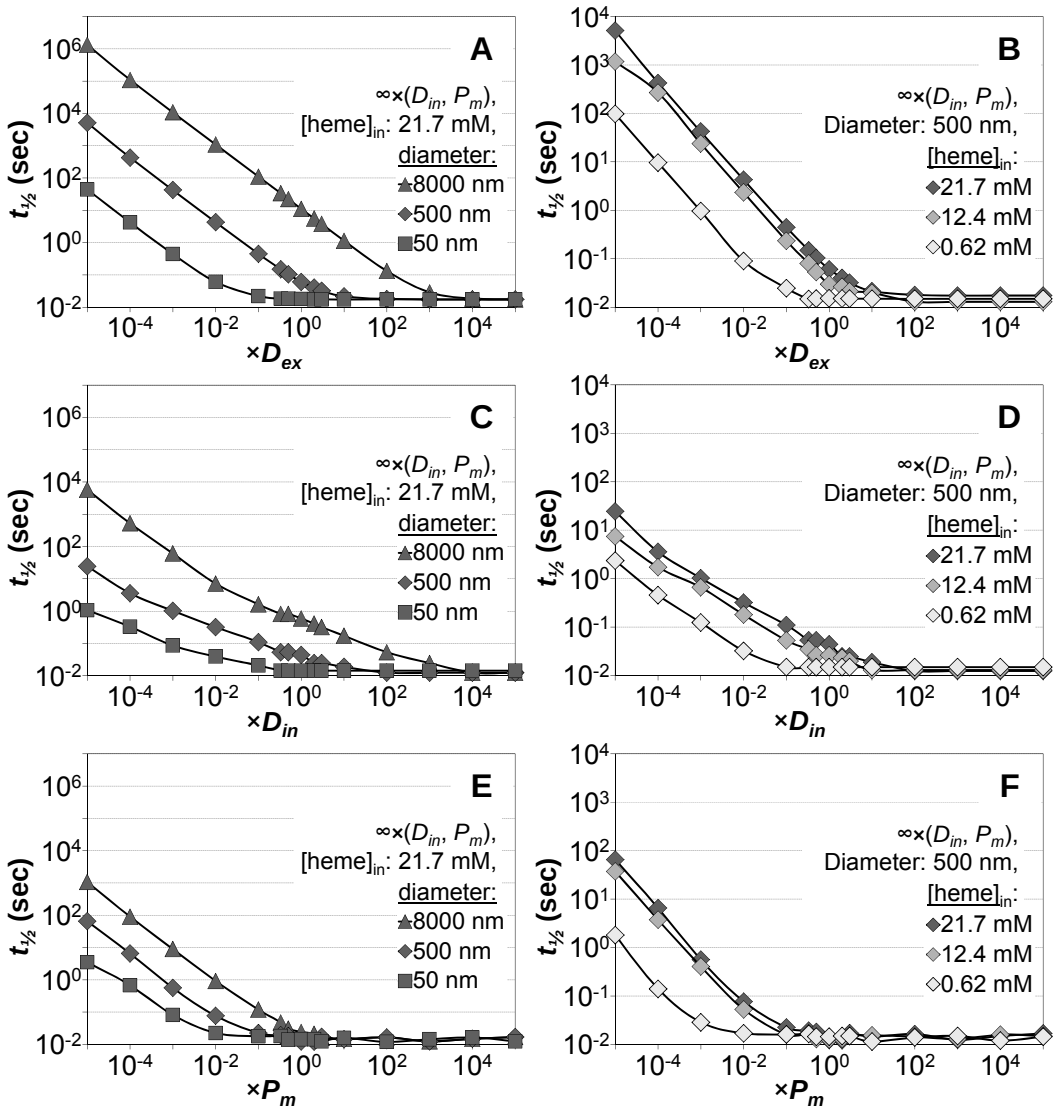
SUPPLEMENTARY MATERIAL

MECHANISMS OF SLOWER NITRIC OXIDE UPTAKE BY RED BLOOD CELLS AND OTHER HEMOGLOBIN CONTAINING VESICLES

Ivan Azarov[1,2,3], Chen Liu[1], Hannah Reynolds[1], Zaharo Tsekouras[1], Janet S. Lee[3,4], Mark T. Gladwin[3,4] and Daniel B. Kim-Shapiro[1*]

From the Departments of [1]Physics and [2]Computer Science, Wake Forest University, Winston-Salem, NC 27109, [3]Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA, 15213, [4]Department of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15213

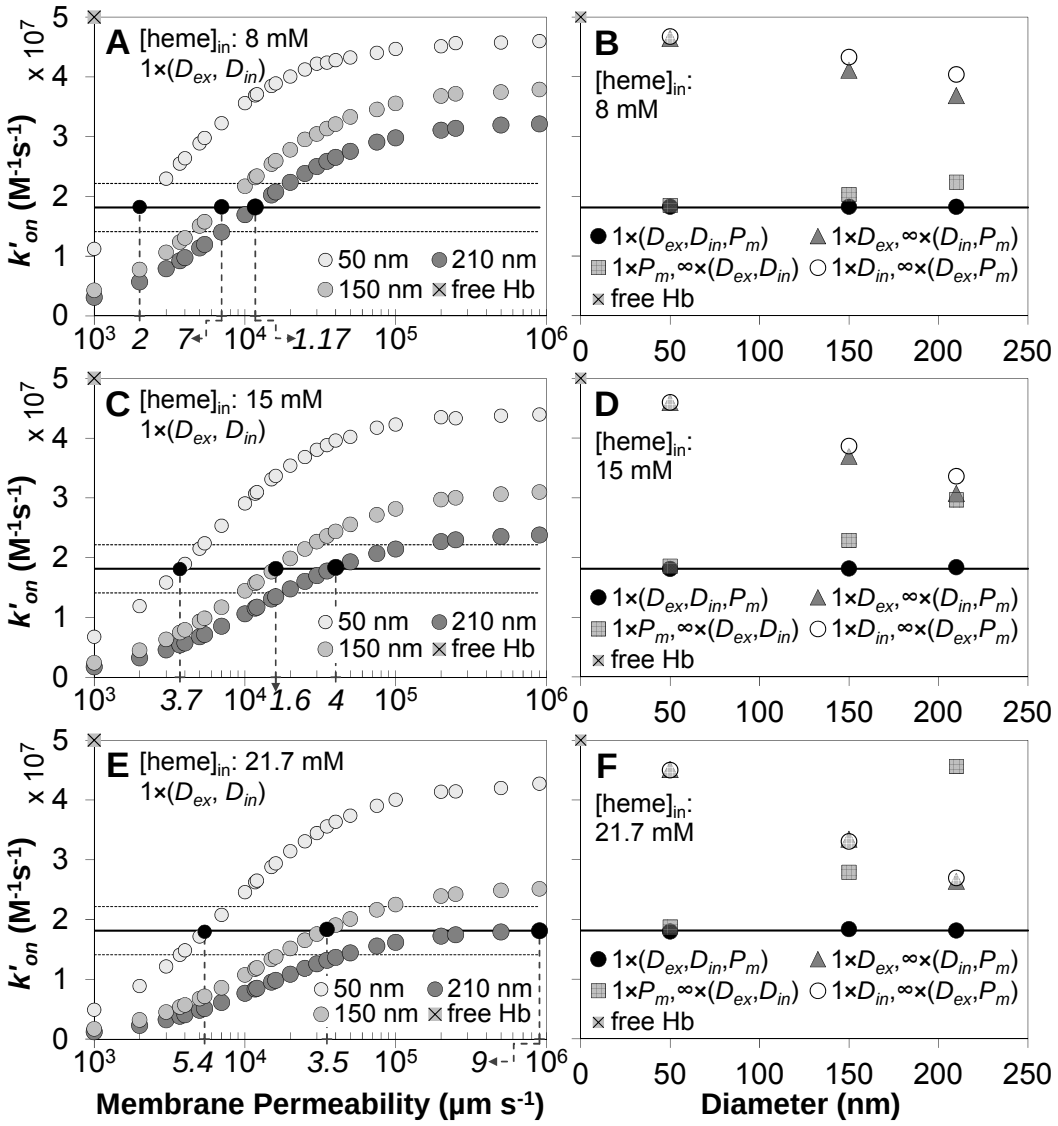
Supplementary Figure 1



Supplementary Fig. 1. Dependence of half-lives in the reaction between NO and deoxygenated phospholipid vesicles on extra and intracellular diffusion and on membrane permeability of NO. *A.* The half-lives (y-axis, logarithmic scale) of reactions between NO and vesicles of three different diameters and with 21.7 mM of intracellular hemoglobin (Hb) plotted vs. the multiplication factor of the physiological value of NO diffusion rate outside of a vesicle (D_{ex}, x-axis, logarithmic scale). For example, the x-axis factor of 10^0 corresponds to the physiological extracellular diffusion coefficient given in table one (D_{ex}). In each of the simulations, the values of NO and Hb diffusion rates inside of a vesicle (D_{in}) and of membrane permeability of NO (P_m) were effectively infinite (Table 1, second-to-bottom row). This is indicated in the legend by multiplying these two parameters with the infinity symbol. *B.* Simulations like those in panel *A*, but for vesicles of one diameter of 500 nm and with three different concentrations of intracellular Hb. *C.* Simulated half-lives for the same vesicles as in panel *A*, but when the diffusion rates of NO and Hb inside of a vesicle (D_{in}) were varied synchronously by fifteen different factors (x-axis) while the diffusion rate outside and membrane permeability of NO were effectively infinite, as indicated with a multiplication factor of infinity for these two parameters in the legend. *D.* Simulations like those in panel C, but for vesicles of one diameter of 500 nm and with three different concentrations of intracellular

Hb. *E*. Simulated half-lives for the same vesicles as in panel *A*, but when the membrane permeability of NO (P_m) was varied by fifteen different factors (x-axis) while the diffusion rates inside and outside were effectively infinite (as indicated by multiplying with the infinity symbol in the legend). *D*. Simulations like those in panel *E*, but for vesicles of one diameter of 500 nm and three different concentrations of intracellular Hb.

Supplementary Figure 2



<u>Supplementary Fig. 2.</u> Simulated apparent bimolecular rate constants for NO uptake by oxygenated red blood cell microparticles. *A* & *C* & *E*. The simulated rate constant (y-axes) for vesicle diameters of 50, 150 and 210 nm plotted vs. microparticle membrane permeability (P_m, x-axes, logarithmic scale) for intracellular hemoglobin (Hb) concentrations of 8 (*A*), 15 (*C*) and 21.7 (*E*) mM. In all of these simulations diffusion of NO in the extracellular space (D_{ex}) and diffusion of NO and Hb inside microparticles (D_{in}) were assigned the physiological values presented in Table 2. This is indicated by the factor of one which multiplies these parameters in the legend. The experimentally measured bimolecular rate constant ($1.81 \pm 0.40 \times 10^7$ M^{-1} s^{-1}) appears as a horizontal black line (in all panels) and its standard deviation as two dotted horizontal black lines. The rate constants which are most similar to the experimentally measured constant at each microparticle diameter are shown as black circles. The membrane permeability values used in simulations in which these constants were obtained are labeled on the x-axis in italic font in their division of the power of ten. *B* & *D* & *F*. Simulated bimolecular rate constants plotted vs. microparticle diameter for intracellular Hb concentrations of 8 (*B*), 15 (*D*) and 21.7 (*F*) mM. Rate constants obtained from simulations when diffusion of NO outside (D_{ex}), diffusion of NO

and Hb inside (D_{in}) and microparticle membrane permeability of NO (P_m) were assigned physiological values are represented by black circles. These are the same simulations that are represented by black circles in the corresponding left panels and the physiological membrane permeability is the one marked on the x-axis of corresponding simulations in the left panels. Rate constants from simulations in which only physiological values of membrane permeability (P_m) were used when diffusion rates (D_{ex}, D_{in}) were effectively infinite (Table 2) are marked as gray squares. In the legend this is indicated by multiplying the symbol for permeability with a one and the symbols for diffusion with the infinity symbol. Rate constants from simulations where only diffusion of NO in the extracellular space (D_{ex}) had a physiological value while the other parameters were effectively infinite are shown as gray triangles. The rate constants from simulations with physiological values of NO and Hb diffusion only inside microparticles (D_{in}) are displayed as white circles. The bimolecular rate constant for the reaction between free oxygenated Hb and NO is shown as a gray square with a black cross on the y-axis of every panel.