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Supplemental Information

Deoxygenation Reduces Sickle Cell Blood Flow at Arterial Oxygen Tension

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Qualitative Assessment of Hemoglobin Oxygen Saturation and Desaturation

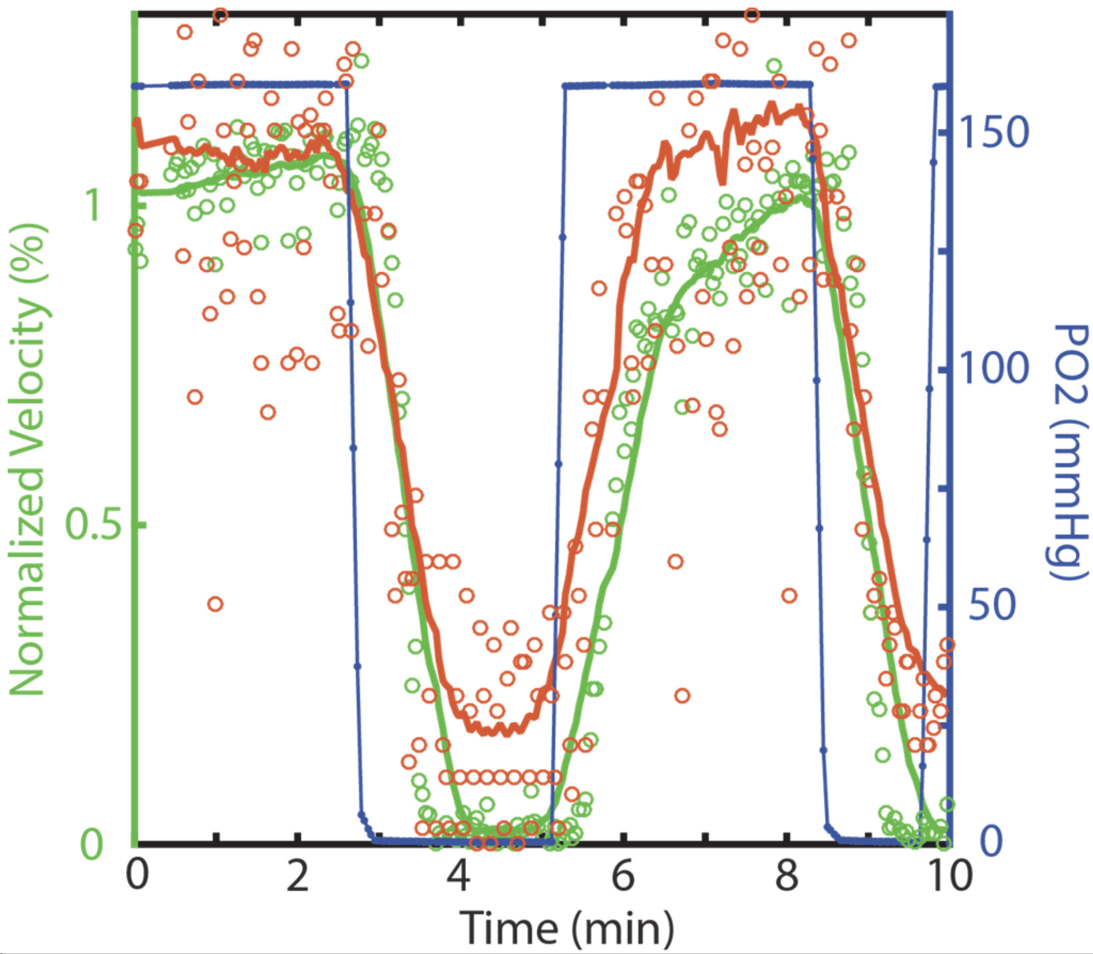

Figure S1. Qualitative Assessment of Hemoglobin Oxygen Saturation and Desaturation. The blue dots and curve (median) show oxygen tension (right-side y-axis) in the gas channel. (See Figure 1 in the main text for a schematic.) The red dots and curve (median) show optical transmission intensity (arbitrary units) through RBCs illuminated at 434 +/- 17 nm, an absorption peak for deoxygenated hemoglobin (1). Transmission intensity correlates with hemoglobin oxygen saturation, with highest intensity corresponding to complete hemoglobin oxygen saturation, and lowest intensity corresponding to complete hemoglobin oxygen desaturation. The green dots and curve (median) show flow velocity as determined by video tracking. A change in oxygen concentration is shortly followed by hemoglobin desaturation and then flow deceleration. Flow stops entirely shortly after the intensity reaches its minimum, corresponding to complete deoxygenation of hemoglobin.

Calibration and Simulation of Oxygen Diffusion Times

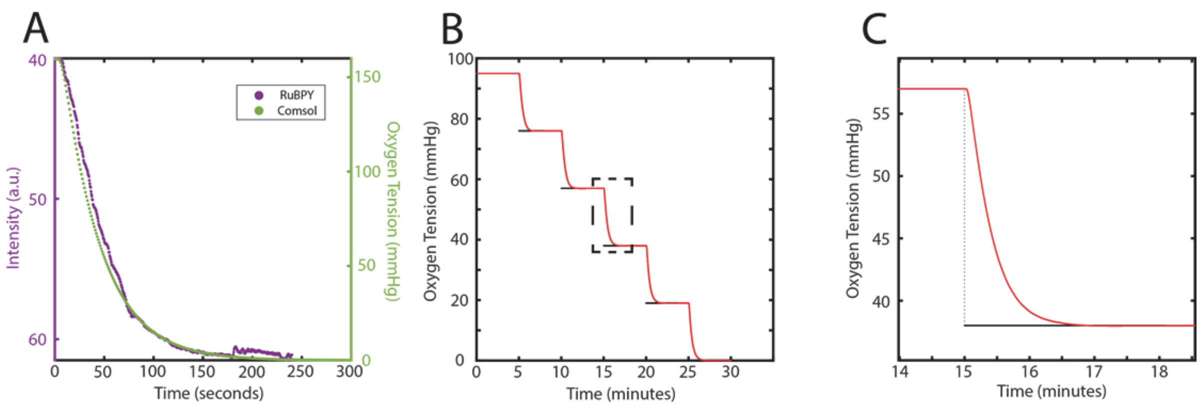

Figure S2. Calibration and Simulation of Oxygen Diffusion Times. Our microfluidic device couples an oxygen gas reservoir to blood microchannels diffusively. See Figure 1 in the main text for a schematic. Here we measure and simulate the diffusion times for oxygen in our system. (**A**) Inverted intensity values for the oxygen sensitive Ru(BPY) dye are shown in purple, and theoretical COMSOL-modeled oxygen tension within the blood channel in green as oxygen tension in the gas channel is switched from 159.6mmHg to 0mmHg. Parameters used for the COMSOL model include a PDMS diffusion coefficient of 8.5×10^{-6} cm^2/s, which was determined via calibration specific to our device, and a water diffusion coefficient of 2.5×10^{-5} cm^2/s for the blood and hydration channels (2). Additionally, we set a no-flux boundary condition at the base of the device to represent the glass slide and set a constant oxygen tension of 159.6mmHg throughout the top and sides of the PDMS device to mimic the oxygen tension of the surrounding environment. Initial oxygen tension was set to 159.6mmHg throughout the device before oxygen tension in the gas channels only were switched to 0mmHg. Measurement and simulation both show that steady state is reached well within the 5 minute duration of each oxygen tension step in our experiments. (**B**) A COMSOL model of oxygen tension within the microfluidic device shows the expected relationship between oxygen tension set in the gas channel (black horizontal lines) and oxygen tension in the blood channels of the device (see schematic in Figure 1). (**C**) A magnified section (dash-outlined section from B) shows that the oxygen tension in the blood channel will equilibrate to the set oxygen tensions in the gas channels during the 5 minutes of each oxygen step.

Transient Deoxygenation Overshoots Do Not Alter Apparent Viscosity

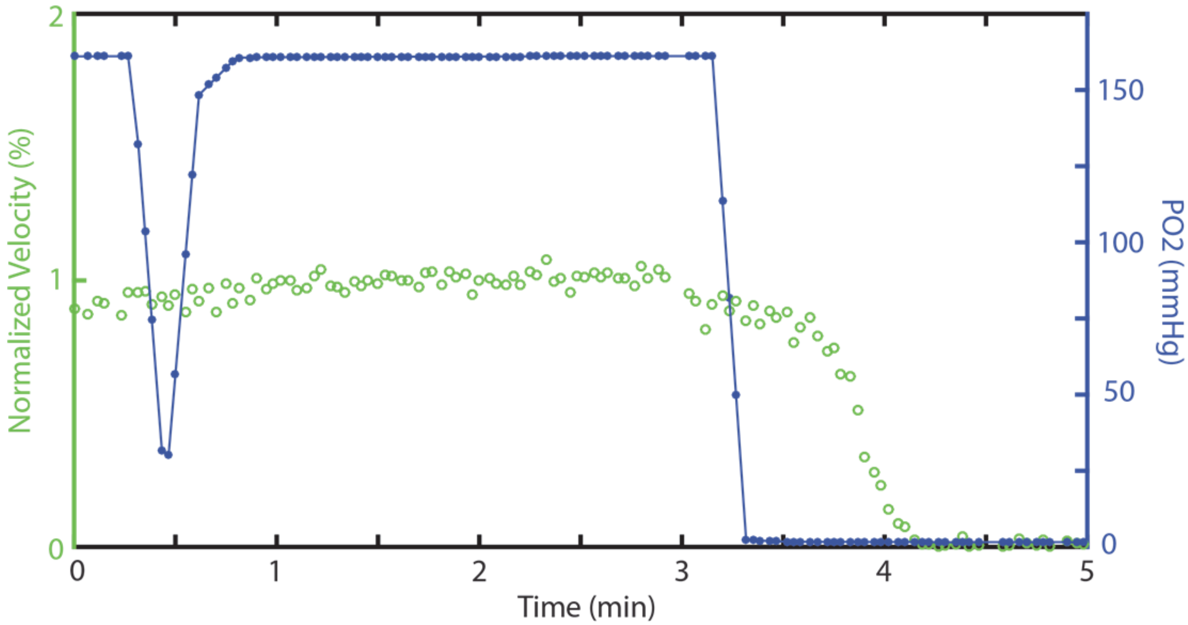

Figure S3. Deoxygenation Overshoots Do Not Alter Apparent Viscosity. Our oxygen mixing system occasionally contains deoxygenation overshoots before recovering to the set oxygen tension. We simulated deoxygenation overshoots by lowering the oxygen tension (blue) for 3 seconds. These quick deoxygenation overshoots did not affect the normalized flow velocity (green) whereas longer drops of oxygen tension dramatically increase apparent viscosity as described and analyzed in the main text.

Apparent Viscosity is Oxygen-Dependent even at Very Low HbS%

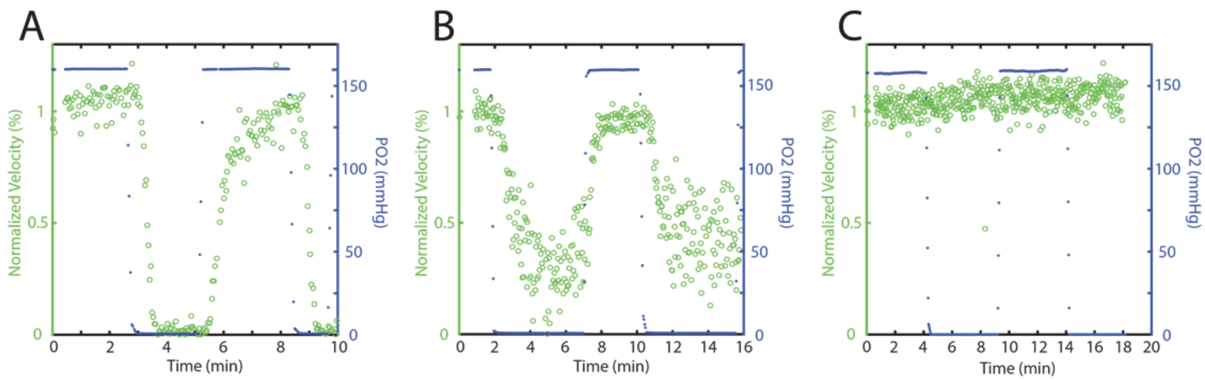

Figure S4. Apparent Viscosity is Oxygen-Dependent even at Very Low HbS%. We measured the effect of very low HbS% on blood apparent viscosity by mixing crossmatch-compatible normal blood with blood from a sickle cell patient. (**A**) Blood flow velocity (green) was measured under controlled oxygen tension (blue). Oxygen tension was cycled from fully oxygenated conditions (159.6mmHg) to fully deoxygenated conditions (0mmHg). The normalized flow velocity of an HbS blood sample (84% HbS) is dependent on oxygen tension and stops flowing when deoxygenated. (**B**) A 10/90 mixture of the same sickle cell blood sample and a crossmatch-compatible normal HbA blood sample has a final HbS% of 8.4 and its apparent viscosity is still oxygen-dependent. (**C**) The apparent viscosity of the normal HbA blood sample is independent of oxygen tension.

References

1. Zhujun, Z., and W. R. Seitz. 1986. Optical sensor for oxygen based on immobilized hemoglobin. Anal. Chem. 58:220-222.
2. Han, P., and D. M. Bartels. 1996. Temperature dependence of oxygen diffusion in H2O and D2O. J. Phys. Chem. 100:5597-5602.