

Supplementary Materials for

Fluorescence lifetime measurement of prefibrillar sickle hemoglobin oligomers as a platform for drug discovery in sickle cell disease.

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This PDF file includes: Figs. S1 to S6

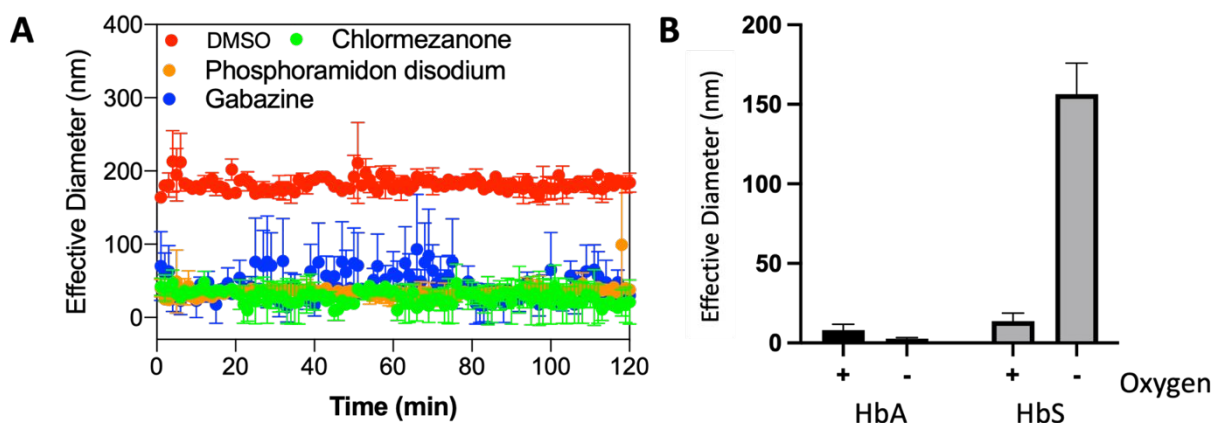


Figure S1: DLS characterization of HbS oligomers. (A) Temporal stability of purified HbS under deoxy conditions. Purified HbS samples (25 μ M) were incubated separately with hit compounds (100 μ M) or DMSO only for two hours at room temperature and degassed with argon for five minutes, and then DLS measurement were taken at room temperature for two hours. (n=2) (B) Oligomer size of HbS and HbA (25 μ M) measured by DLS in the presence or absence of oxygen and in the presence of DMSO.

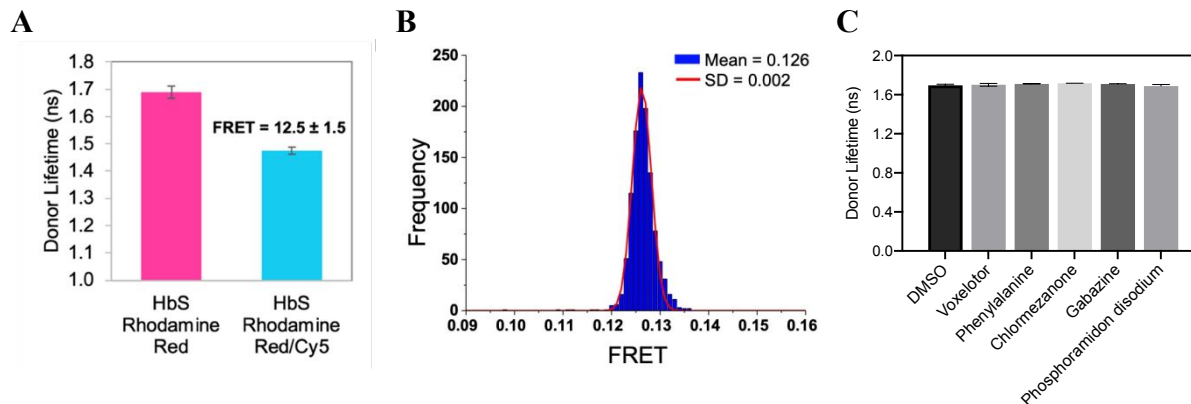
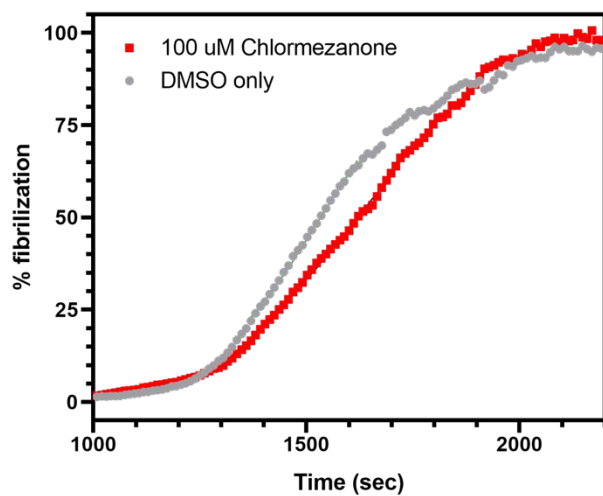


Figure S2: Lifetime FRET. (A) Fluorescence lifetime measurements with HbS-rhodamine red (donor only) and HbS-rhodamine red HbS-Cy5 (FRET pair). The FRET efficiency of the HbS samples were calculated from lifetimes using Eq.1 (B) Histogram plots of all compounds from the LOPAC screen after removal of fluorescent compounds show an average FRET efficiency of 0.126 ± 0.002 . (C). Fluorescence lifetime measurements with HbS-rhodamine red (donor only) in presence of DMSO, controls and three lead compounds.



	Slope (% fibrilization/sec)	R ²
DMSO	0.1727	0.9993
20 uM Chlormezanone	0.1626	0.9985
100 uM Chlormezanone	0.1359	0.9973
20 uM Gabazine	0.1359	0.9977
100 uM Gabazine	0.1527	0.9957

Figure S3: Fibrillization Assay. Effect of hit compounds on polymerization of deoxyHbS in high potassium phosphate buffer. Curves represent growth of polymer as a function of time in the presence or absence of drug. Table shows slopes of lines fitted to linear part of the growth curve (approximate linear growth rate) for each condition as well as quality of fit.

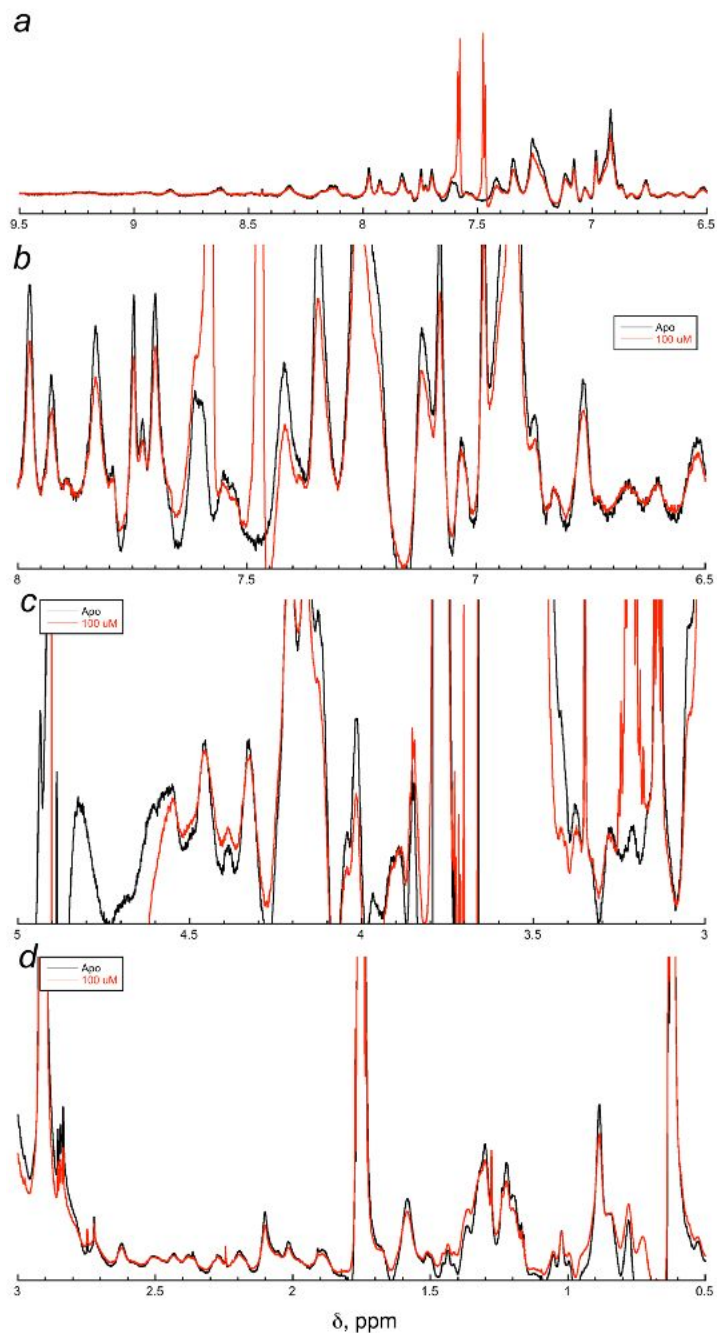
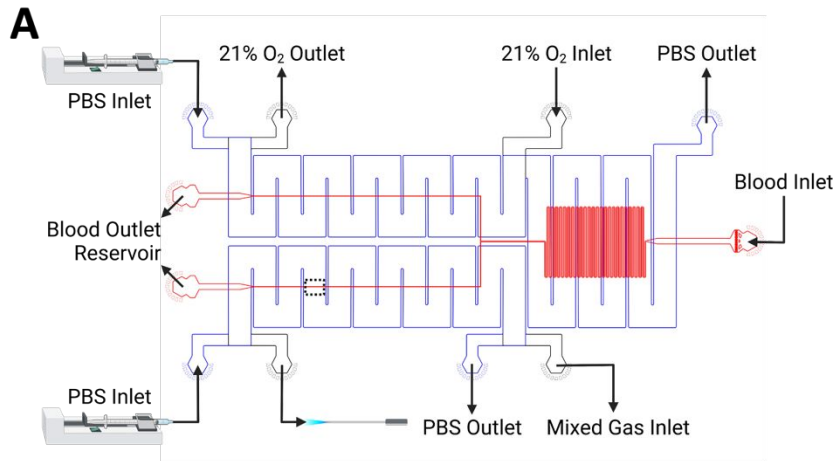


Figure S4: Chlormezanone binding confirmed by NMR. Superimposed 1D ^1H NMR spectra showing chemical shift changes to HbS upon chlormezanone binding. The HbS (50 mM) only control is shown in black and HbS (50 mM) with chlormezanone (100 μM) is shown in red. Chemical shift perturbations observed upon binding chlormezanone in (A) Amide region; (B) aromatic and amide region region; (C) aliphatic region; and (D) methyl region. NMR signals attributed to free ligand are highlighted with asterisks. All spectra were collected on a Bruker Avance 900 MHz NMR Spectrometer at 288 K.



B Transverse View

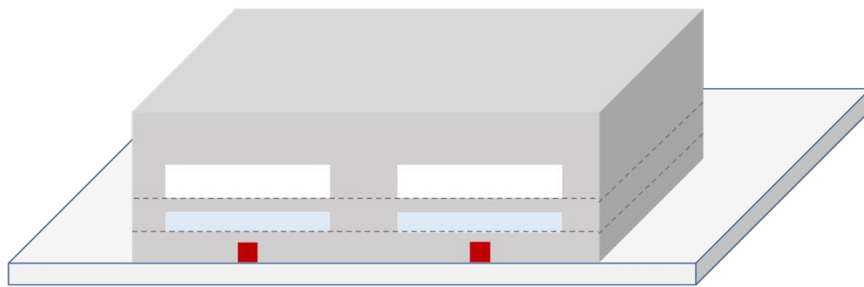


Figure S5: Schematic of microfluidic device for measuring sickle blood flow. (A) Microfluidic device consisting of three polydimethylsiloxane (PDMS) layers: blood (red), hydration (blue), and gas (black). High-speed cameras capture images to track blood flow in the region of interest (black, dashed box). (B) Transverse view of the multi-layered microfluidic device bonded to a glass slide.

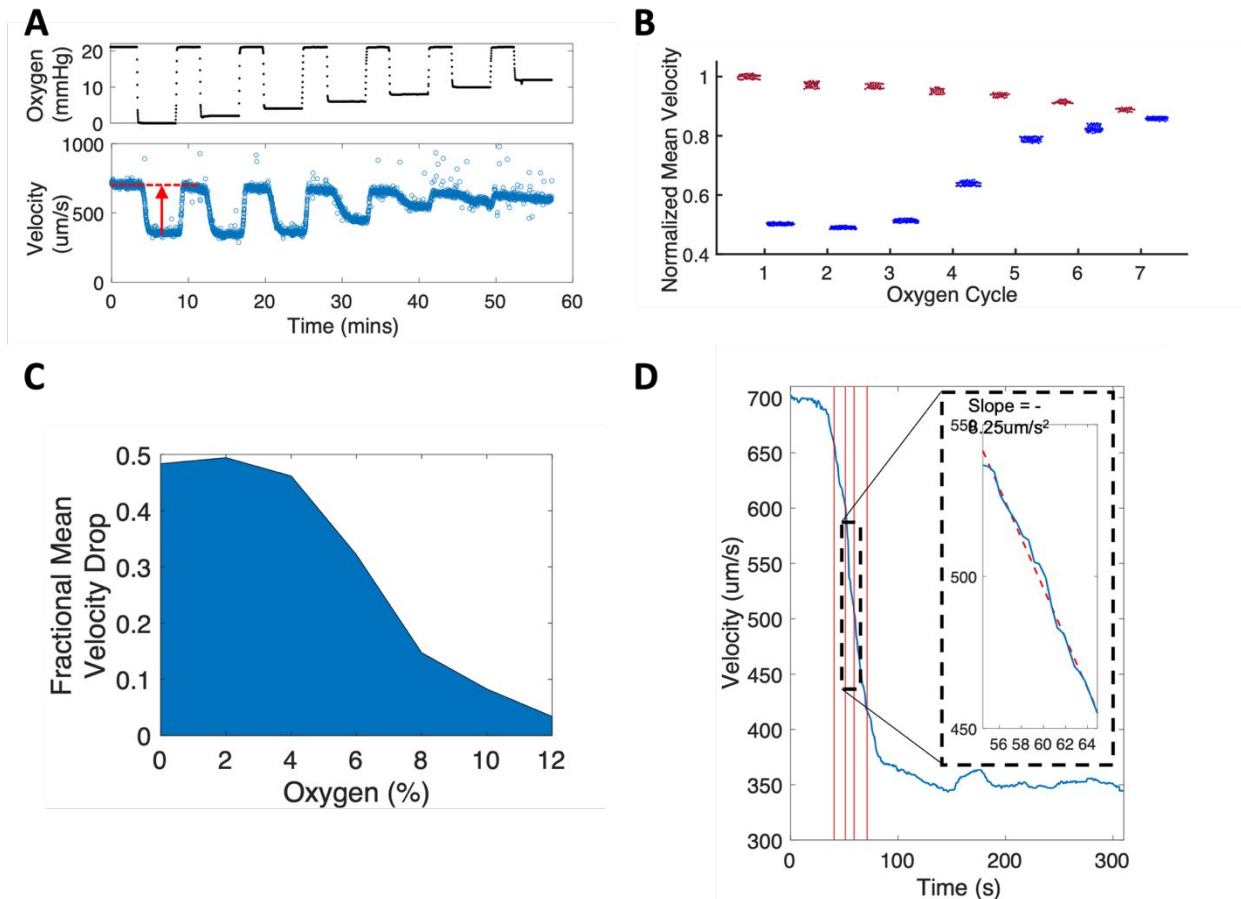


Figure S6: Quantification of sickle blood flow. (A) Raw oxygen and velocity recordings during microfluidic experiment. Red arrow indicates the change in the steady state mean velocity after introducing hypoxic conditions on the microfluidic chip. (B) The on-chip oxygen concentration is cycled seven times during the experiment between 21% O_2 (Red) and hypoxia (Blue). Changes in the velocity during each cycle are normalized to the average velocity of the initial 21% cycle. (C) Shaded region represents the area under the curve (AUC) using the steady state mean velocity changes at each oxygen concentration. This measurement quantifies the overall oxygen response of the blood by accounting for both the magnitude of the steady state mean velocity drop as well as the persistence of oxygen-dependence at higher oxygen concentrations (D) Blood deceleration is measured by a segmented moving mean of the velocity response during the hypoxic transition using Matlab signal processing. Inset shows moving mean (blue) and linear curve fit (red, dashed) to determine the slope of the signal.