

Figure S1. The stoichiometry of liberation of NO from PROLI/NO. Upper panel:

Concentration of consumed oxygenated myoglobin (oxyMb) calculated from absorption at 582 nm using the extinction coefficients for oxyMb and metMb, after addition of PROLI/NO (1 mM in 0.1N NaOH) to deoxygenated solution of oxyMb (50μM). The amount of NO derived from PROLI/NO is equal to the amount of oxyMb consumed ([oxyMb]₀ – [oxyMb]) due to conversion to metMb. **Lower panel:** Concentration of NO delivered from PROLI/NO (stock 1 μM) dissolved in deoxygenated PBS/DTPA that was injected into 0.1 N NaOH solution in the reaction chamber of NO analyzer. NO was quantified based on detector response established with GSNO.

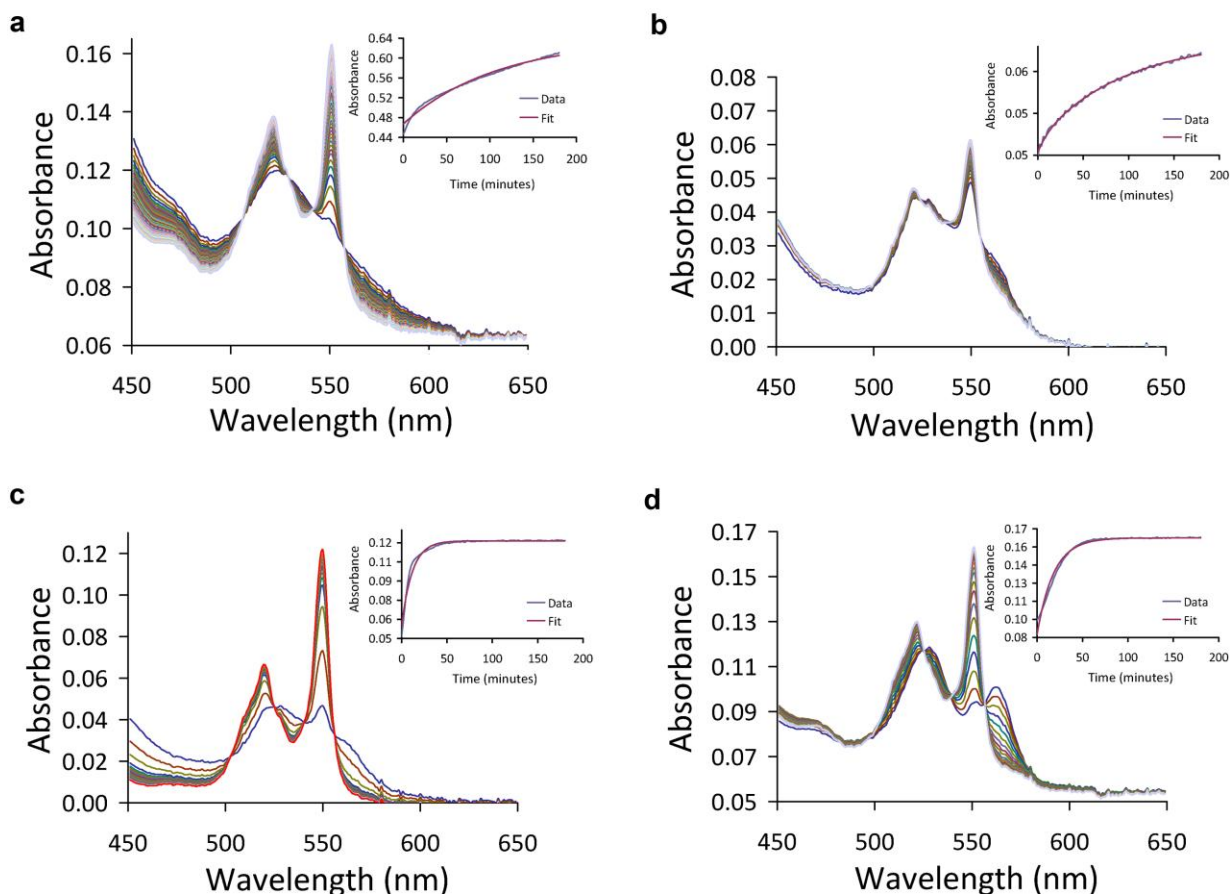


Figure S2. GSH/NO-dependent reduction of horse heart ferric cytochrome c (C-7752) purified by sedimentation and filtration. Time-resolved absorption spectra following combination of cyt c (50 μM), (a) GSH (1 mM), (b) PROLI/NO (25 μM), (c) GSH (1 mM) and PROLI/NO (25 μM) or (d) GSH (1 mM) and PROLI/NO (500 μM) under anaerobic conditions at room temperature and pH 7.4. Spectra were collected every two minutes following mixture of the reagents in a 0.1 cm pathlength cell. Insets show kinetic traces of absorption at 550 nm and the theoretical fit to a single exponential decay with observed rate constants of $0.16 \times 10^{-3} \text{ s}^{-1}$, $1.4 \times 10^{-3} \text{ s}^{-1}$, and $0.92 \times 10^{-3} \text{ s}^{-1}$ for conditions in (a), (c) and (d) and the theoretical fit to a double exponential decay with observed rate constants of $1.5 \times 10^{-3} \text{ s}^{-1}$ and $0.17 \times 10^{-3} \text{ s}^{-1}$ for conditions in (b) [fit to a single exponential process did not work well for these data]. Notably, and similar to the data shown in Figure 1, combining GSH and NO reduces cyt c much faster than when only one of these reagents is present (compare kinetic traces in (c) and (d) to (a) and (b)). In addition, the reduction is actually slower at higher [NO] than lower [NO] (compare (c) and (d)). Finally, as in Figure 4, combination of GSH and NO with ferric cyt c results in very efficient GSNO formation; $10.2 \pm 0.2 \mu\text{M}$ GSNO was measured after 20 minutes for the reaction performed with GSH (1 mM) and PROLI/NO (25 μM) as in panel (c).