

Supporting Information

Mechanism of NO binding to soluble guanylyl cyclase: implication for the second NO binding to the heme proximal site

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Running title: NO binding mechanism to soluble guanylyl cyclase

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Fig. S1. Kinetics of NO binding to sGC. NO binding was determined by mixing 5 μM sGC and NO solution at 25 - 500 μM range at 25 $^{\circ}\text{C}$. Only those data that satisfy true pseudo-first order conditions are shown. The smooth red traces are the fits to a one-exponential function. The beginning < 2 ms plateau regions are the data before flow stopped, and is in the dead time of the stopped-flow instrument. The secondary plot between observed rate and $[\text{NO}]$ in the inset was used to determine the k_{on} and k_{off} rate constants to be: $1.1 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ and 24.7 s^{-1} , respectively.

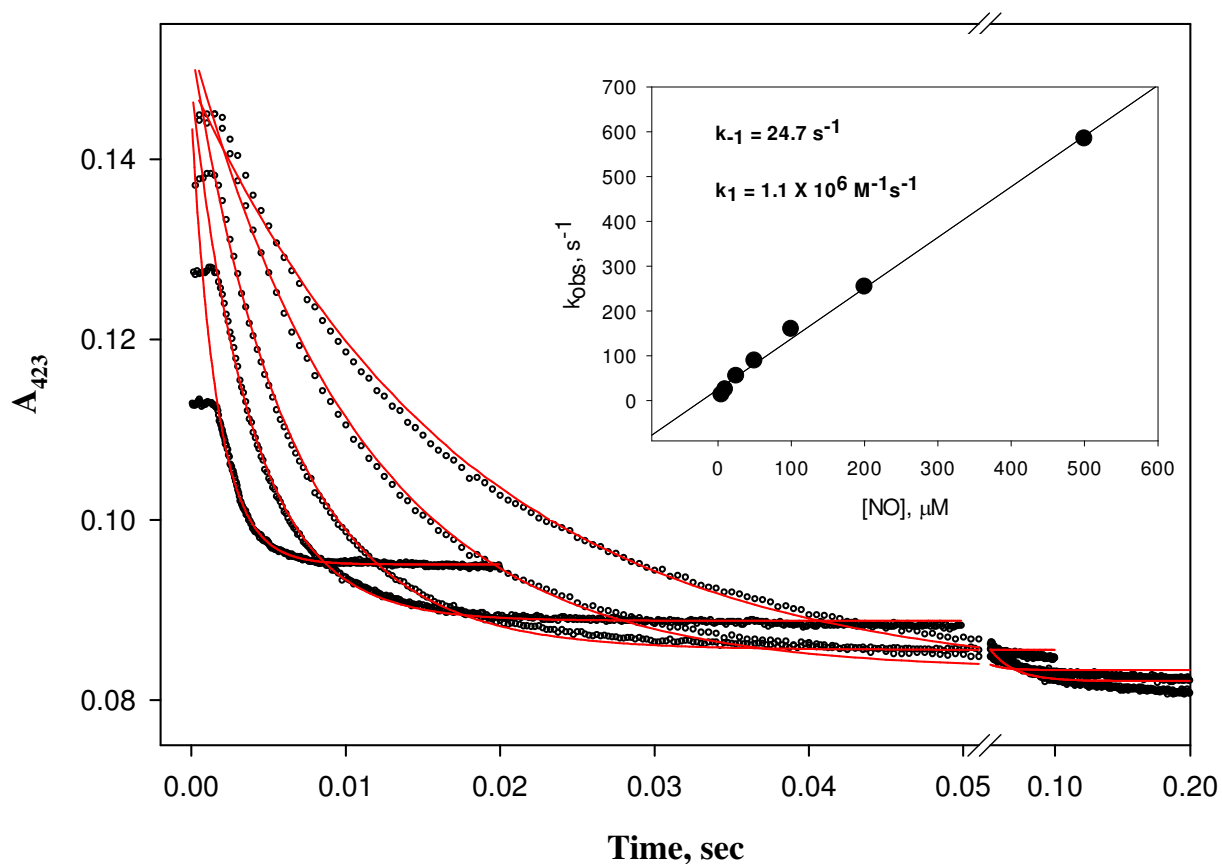


Fig. S2. Effect of anaerobic thawing/freezing on the sample trapped by sequential mixing freeze-trap EPR. EPR from samples shown in Fig. 2A and 2B ((a) and (c)) and spectra recorded after anaerobic thawing these two samples at 24 °C for 1 min and refreezing ((b) and (d)). Each spectrum was tested to be a composite of ~ 55 and ~43% of the $^{15}\text{NO-sGC}$, respectively.

