Supplementary Information for:

Reaction between a haemoglobin model compound and hydrogen sulfide in aqueous solution

Kenji Watanabe,[a,*] Toshikane Suzuki,[b] Hiroaki Kitagishi,[b] Koji Kano[b,*]

[a] *Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan. E-mail: kwatanabe@phar.kyushu-u.ac.jp*
[b] *Department of Molecular Chemistry and Biochemistry, Faculty of Science and Engineering, Doshisha University, Kyotanabe, Kyoto 610-0321, Japan. E-mail: kojikano1223@gmail.com*

Instruments.

UV-Vis absorption spectra were measured using a Shimadzu UV-2400 spectrophotometer. Electron paramagnetic resonance (EPR) spectra were recorded on a JES-TE200 ESR system (JEOL Ltd., Tokyo, Japan). F-52 pH meter (Horiba Ltd., Kyoto, Japan) was used for pH measurements.

Materials.

Water was distilled by Yamato Auto Still Glass Model WG250, and further purified by Millipore Simpack 1. NaSH (100 %) was purchased from Strem Chem. Inc. and was used as received. CO (99.999 %), O_2 (99.999 %) and argon (99.999 %) gasses were purchased from Sumitomo Seika Chemicals. HemoCD3 was prepared as described in a previous study.[1] All other reagents were purchased and used as received.

Preparation of NaSH solution.

A solution of NaSH was freshly prepared in the globe box under argon atmosphere. Deoxidized aqueous phosphate buffered solutions were prepared by three freeze-pump thaw cycles and brought into the globe box. The concentration of the NaSH aqueous solution was determined by iodometric titration:[2] 10 mL of aqueous NaSH solution was combined with 5 mL of aqueous I_2 solution (0.05 M) containing appropriate amount of KI (0.1 M) and starch (solution A). Then, aqueous $Na_2S_2O_3$ solution (0.1 M) was titrated for the solution A. The point where the blue solution turned colourless was considered to be the end point of the titration.

Redox reaction between I_2 and I^- is shown in Eq 1.

$$I_2 + 2e^- \rightleftarrows 2I^- \ (E^0 = 0.53\ \mathrm{V}) \qquad \text{Eq 1}$$

Equilibrium between I_2 and I_3^- in the presence of KI is shown in Eq 2.

$$I_2 + KI \rightleftarrows I_3^- + K^+ \qquad \text{Eq 2}$$

NaSH and $Na_2S_2O_3$ reduce I_2 (Eq 3 and 4).

$$I_2 + NaSH \rightarrow S\downarrow + NaI + HI \qquad \text{Eq 3}$$

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_2O_6 \qquad \text{Eq 4}$$

Therefore, the initial concentration of NaSH ($[NaSH]_o$) in solution A is expressed as Eq 5. $[I_2]_o$ and $[I_3^-]_o$ represent the initial concentrations of I_2 and I_3^-, respectively, in solution A. $[I_2]$ and $[I_3^-]$ represent the final concentration of I_2 and I_3^-, respectively, in solution A, where NaSH is fully consumed.

$$[NaSH]_0 = ([I_2]_o + [I_3^-]_o) - ([I_2] + [I_3^-]) \qquad \text{Eq 5}$$

$[I_2]$ and $[I_3^-]$ are expressed as shown in Eq 6,

$$[I_2] + [I_3^-] = x \cdot [Na_2S_2O_3] / 2v \qquad \text{Eq 6}$$

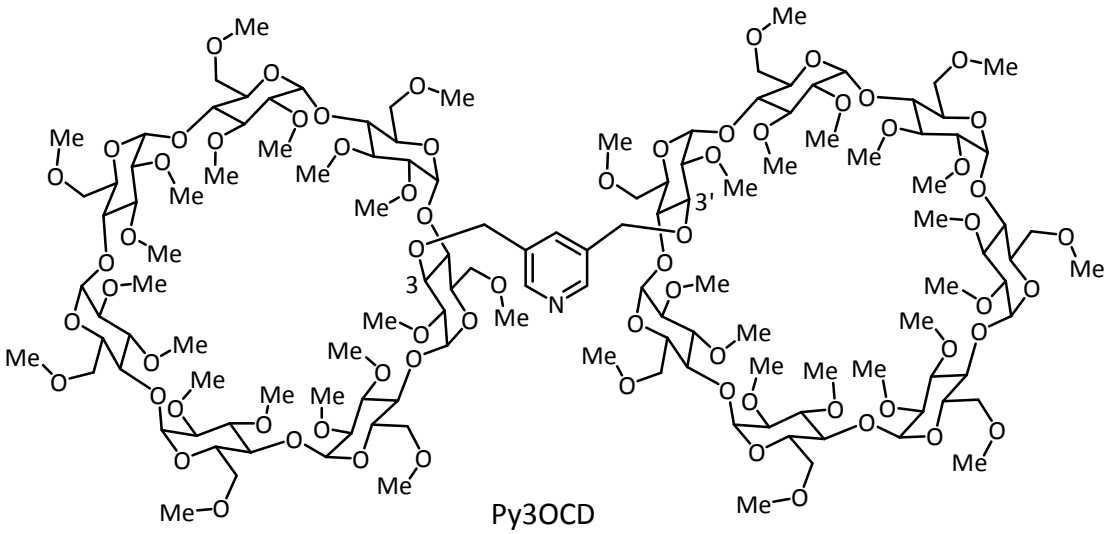
where $[Na_2S_2O_3]$, x, and v represent the concentration of the aqueous $Na_2S_2O_3$ solution, titrated volume of $Na_2S_3O_3$ solution, and the volume of solution A.

Eqs 5 and 6 are combined leading to Eq 7, which gives the $[NaSH]_0$ of the solution A.

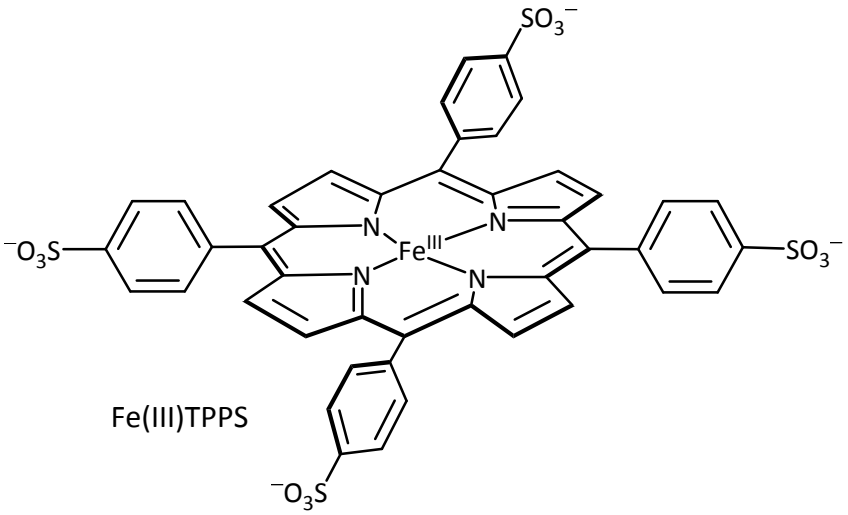
$$[NaSH]_0 = ([I_2]_o + [I_3^-]_o) - x \cdot [Na_2S_2O_3]/2v \qquad \text{Eq 7}$$

1. K. Watanabe, H. Kitagishi and K. Kano, *Angew. Chem. Int. Ed.*, 2013, **52**, 6894-6897.
2. Hujii, H. *Bunsekikagaku*, 1972, **21**, 1574-1579.

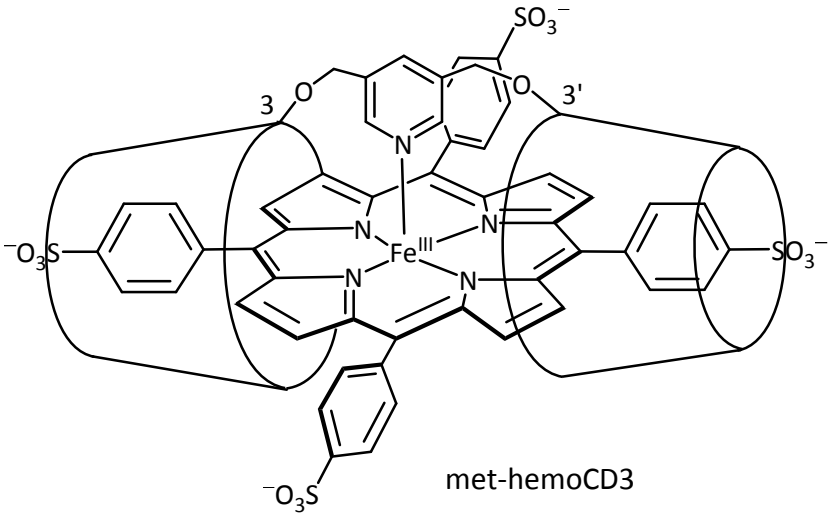
Structures of Py3OCD, Fe(III)TPPS and met-hemoCD3.



Py3OCD



Fe(III)TPPS



met-hemoCD3

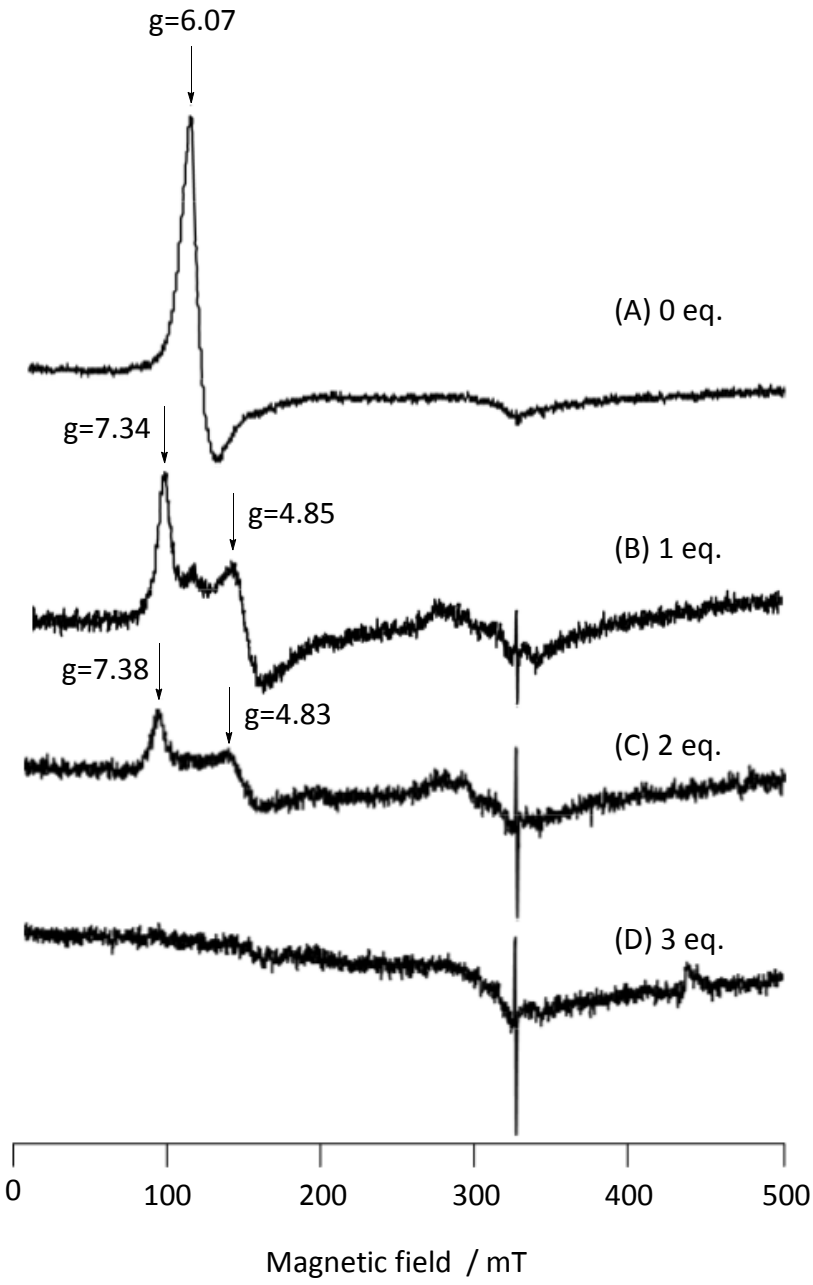


Fig. S1 EPR spectra of the Fe(III)TPPS/(TMe-β-CD)$_2$ complex (5.0×10^{-4} M) in the presence of various amounts of NaSH (0 eq. (A); 1 eq. (B); 2 eq. (C); 3 eq. (D)) in phosphate buffer at pH 6 and 77 K.

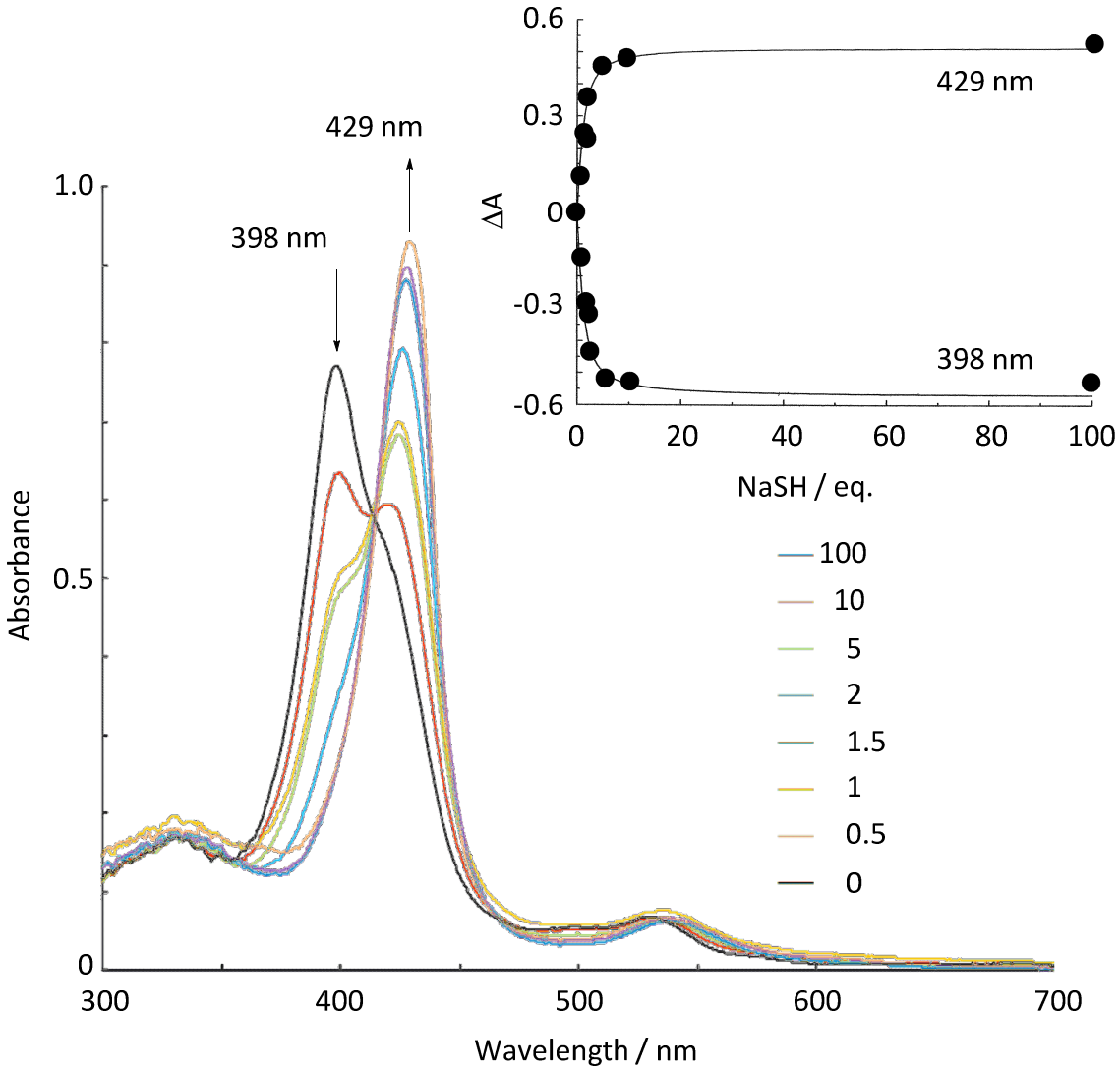


Fig. S2 UV-Vis absorption spectral changes of met-hemoCD3 (5×10^{-6} M) upon addition of NaSH in phosphate buffer at pH 6 and 25 °C.

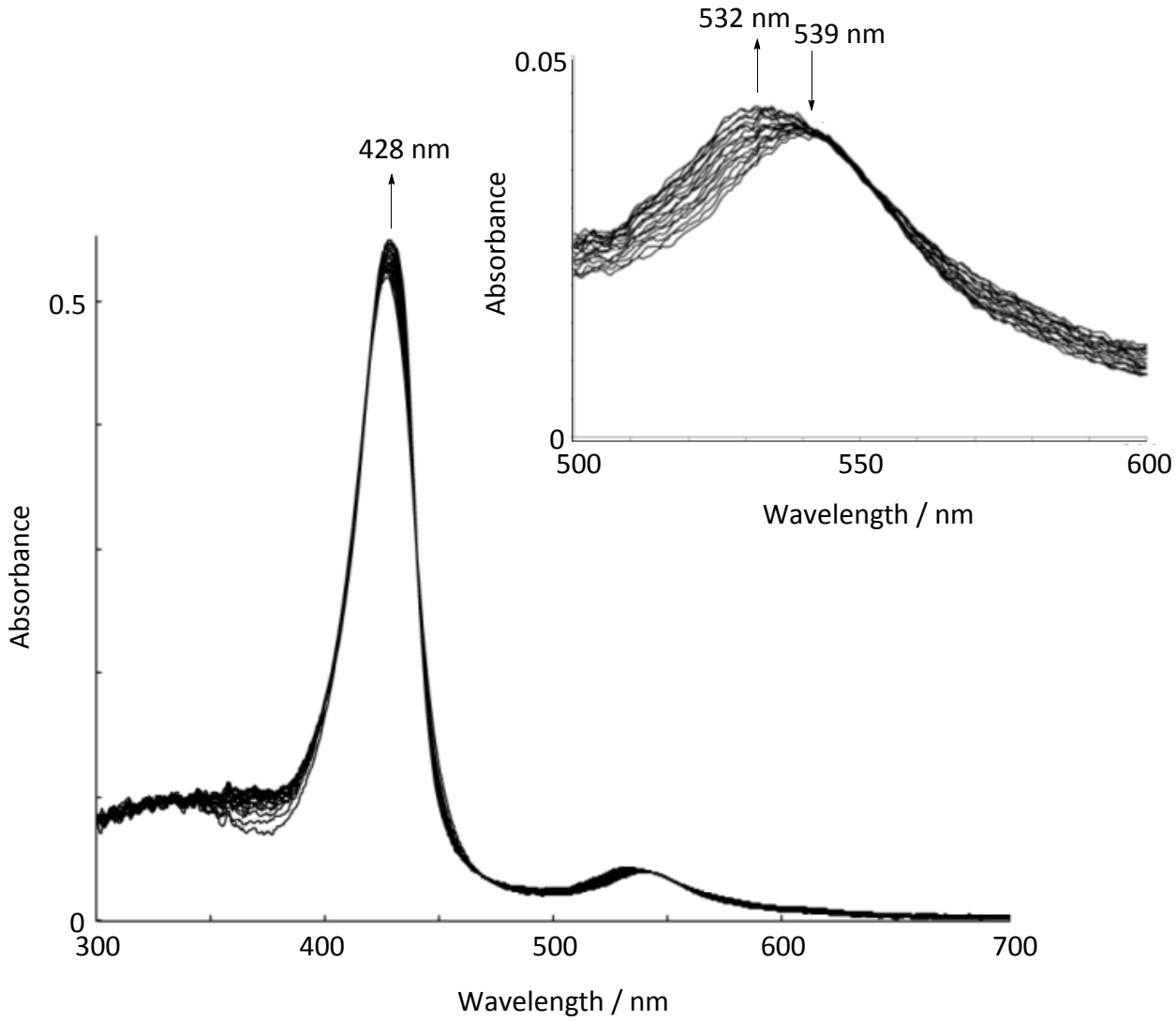


Fig. S3 Progressive absorption spectral changes during spontaneous reduction of (SH^-)-met-hemoCD3 ([met-hemoCD3 = 5.0×10^{-6} M]; [NaSH] = 5.0×10^{-5} M) to Fe(II)-hemoCD3 in deoxidised phosphate buffer at pH 7 and 25 °C. The spectra were recorded at 5-min intervals.

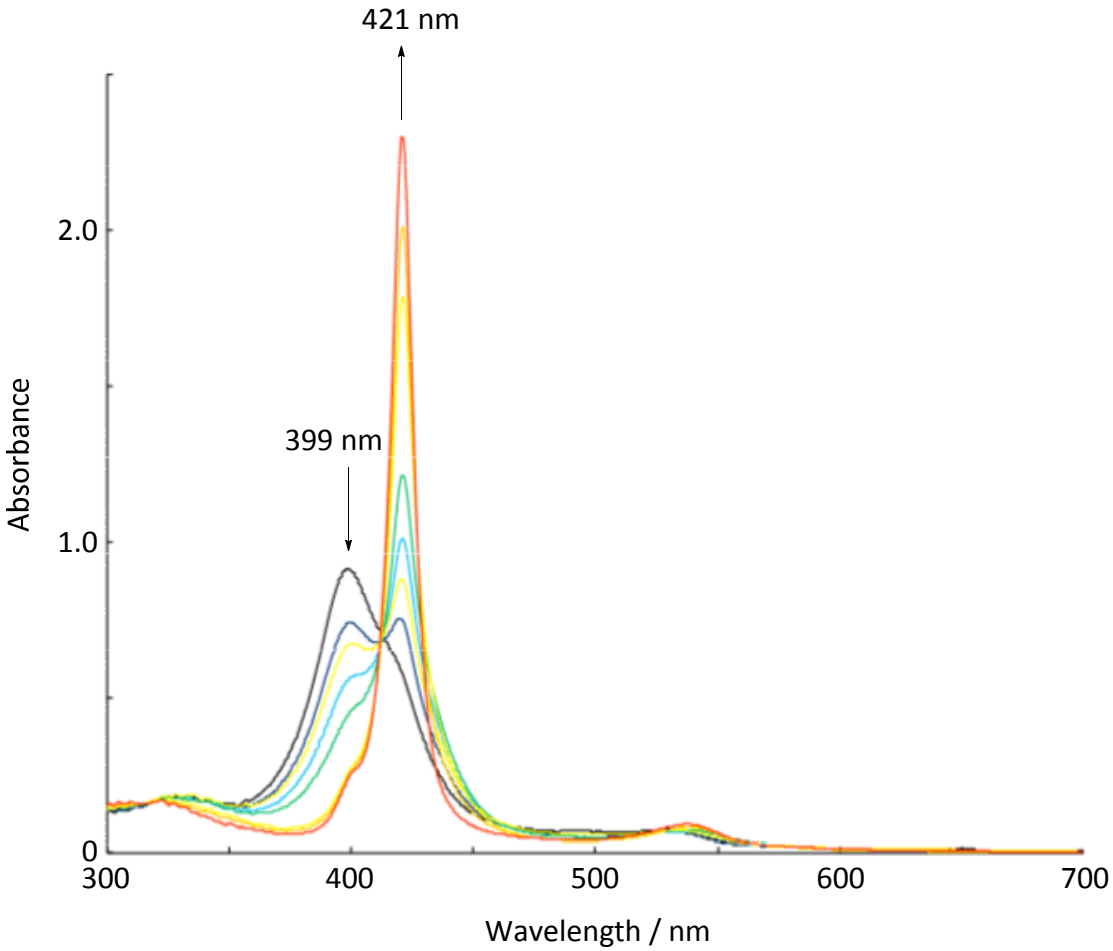


Fig. S4 UV-Vis absorption spectral changes of met-hemoCD3 (5×10^{-6} M) upon addition of NaSH (0-100 eq.) in phosphate buffer at pH 6 and 25 °C under CO atmosphere.