

## Supporting Information

### Interaction of Cob(III)alamins with Hypothiocyanite. Evidence for the Formation of Hypothiocyanitocobalamin

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#### Experimental

Hydroxocobalamin hydrochloride (Sigma-Aldrich; HOCbl; ≥96%), cyanocobalamin (CNCbl; J & K; 98 %), methylcobalamin (MeCbl; J & K; 98 %), lactoperoxidase from bovine milk (LPO; Sigma; ≥ 200 units/mg), potassium cyanate (Sigma-Aldrich; 96%), potassium thiocyanate (Sigma-Aldrich; 99%), lead(II) thiocyanate (Aldrich; 99.5 %), glutathione (GSH; J & K; 99 %) and seleno-D,L-methionine (Sem; Sigma; ≥ 99%) were used without additional purification. Glutathionylcobalamin (GSCbl) was synthesized according to the published procedure.<sup>[1]</sup> 5-thio-2-nitrobenzoic acid (TNB) was prepared according to published procedure.<sup>[2]</sup>

Phosphate buffer solutions (0.1 M; pH 6.3) were used to maintain pH during the measurements. The pH values of solutions were determined using Multitest IPL-103 pH-meter (SEMICO) equipped with ESK-10601/7 electrode (Izmeritelnaya tekhnika) filled by 3.0 M KCl solution. The electrode was preliminarily calibrated using standard buffer solutions (pH 1.65–12.45).

Concentrations of Cbl stock solutions were determined using UV-vis spectroscopy via a conversion of Cbl to its dicyano-form (extinction coefficient is 30400 M<sup>-1</sup>·cm<sup>-1</sup> at 368 nm)<sup>[3]</sup>. Concentrations of SCN<sup>-</sup> and OSCN<sup>-</sup> were determined using their reactions with Fe(III) (0.25 M CF<sub>3</sub>COOH) and TNB (pH 6.3)<sup>[4]</sup>, respectively.

Ultraviolet-visible (UV-vis) spectra were recorded on a cryothermostated (± 0.1 °C) Cary 50 UV-vis spectrophotometer in quartz cells.

Observed equilibrium constant ( $K_{\text{obs}}$ ) for OSCN<sup>-</sup> in the presence of SCN<sup>-</sup> (0.01 M) binding was calculated using eq. (S1).<sup>[5]</sup>

$$A = \frac{A_0 + A_\infty K_{\text{obs}} [\text{OSCN}^-]}{1 + K_{\text{obs}} [\text{OSCN}^-]} \quad (\text{S1})$$

where [OSCN<sup>-</sup>] is hypothiocyanite concentration in solution, M; A is absorbance at the monitoring wavelength for the complex at a particular OSCN<sup>-</sup> concentration; A<sub>0</sub> is the absorbance for the starting complex; A<sub>∞</sub> is the absorbance for the final complex.

Equilibrium constant for the reaction between OSCN<sup>-</sup> and H<sub>2</sub>OCbl ( $K_1$ ) was determined using eq. (S2).

$$K_1 = K_{\text{obs}} \cdot K_2 \cdot [\text{SCN}^-] \quad (\text{S2})$$

where [SCN<sup>-</sup>] is thiocyanate concentration, M;  $K_2$  is equilibrium constant for the reaction between SCN<sup>-</sup> and H<sub>2</sub>OCbl ( $K_2 = 1.4 \cdot 10^3 \text{ M}^{-1}$ )<sup>[6]</sup>.

Experimental data were analyzed using Origin 9.1 software.

## References

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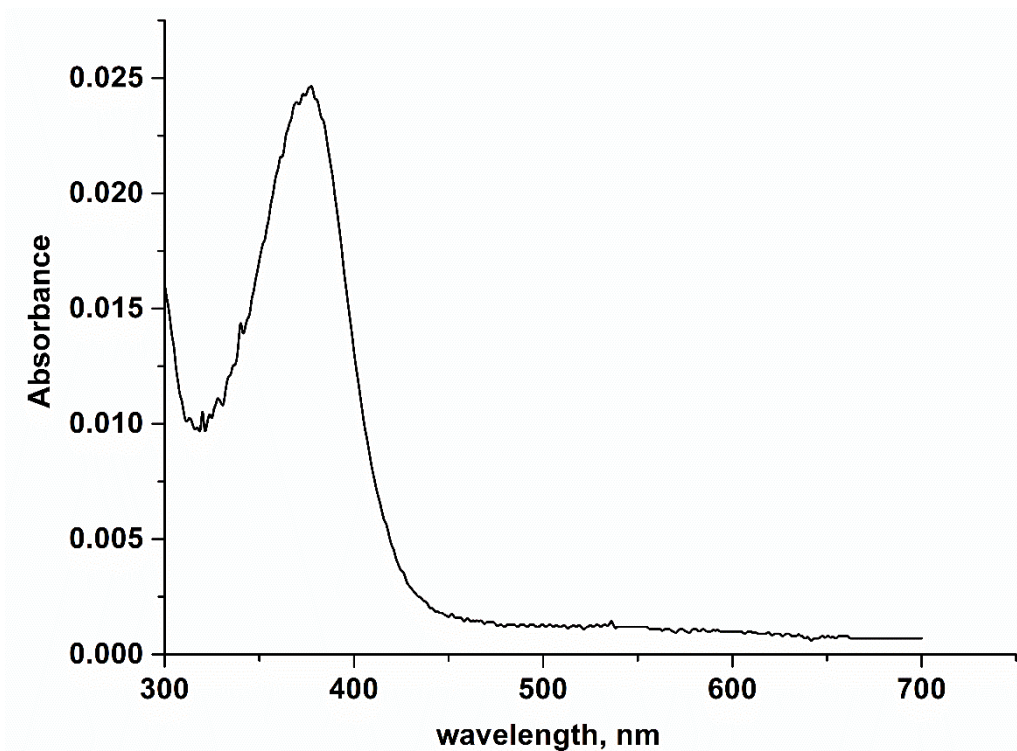


Figure S1. UV-vis spectrum of  $\text{OSCN}^-$  (0.9 mM) generated by mixing of  $\text{SCN}^-$  (5.0 mM) with  $\text{H}_2\text{O}_2$  (1.0 mM) and LPO (ca. 0.6 U/ml) at pH 6.5, 25.0 °C

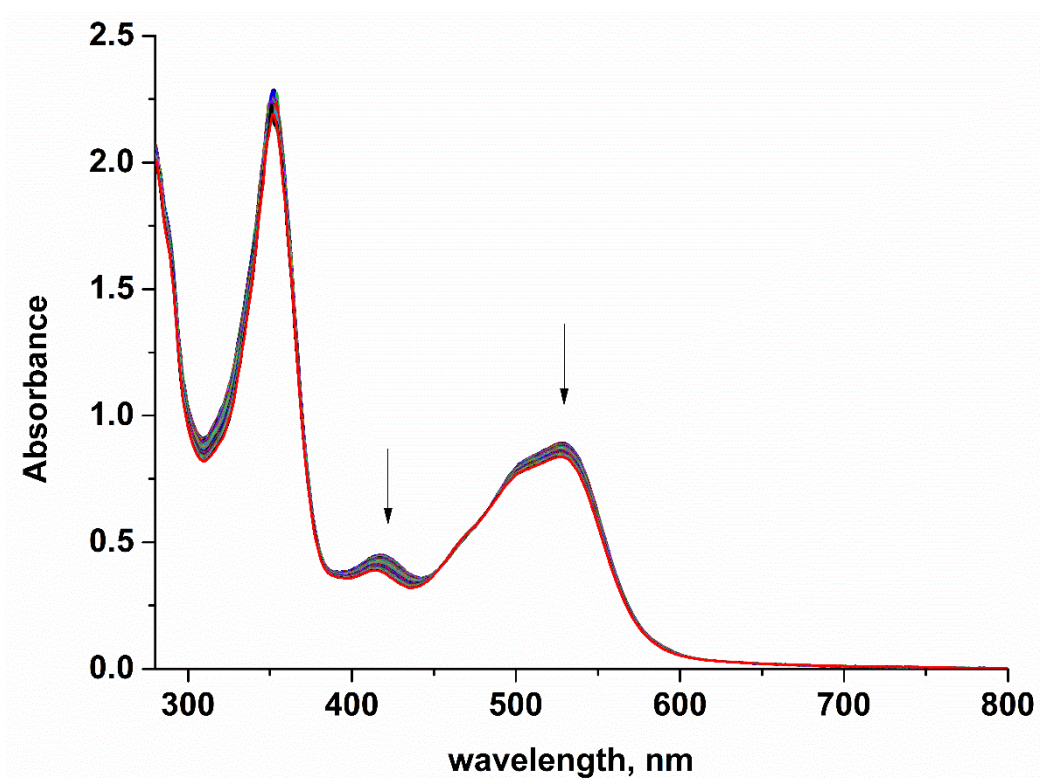


Figure S2. UV-vis spectra of  $\text{H}_2\text{OCl}$  ( $1.0 \cdot 10^{-4}$  M) in the presence of LPO ( $\geq 29$  U/ml) and  $\text{H}_2\text{O}_2$  ( $2.0 \cdot 10^{-3}$  M) at 37.0 °C. Time interval between spectra is 10 s, spectra were recorded for 40 min

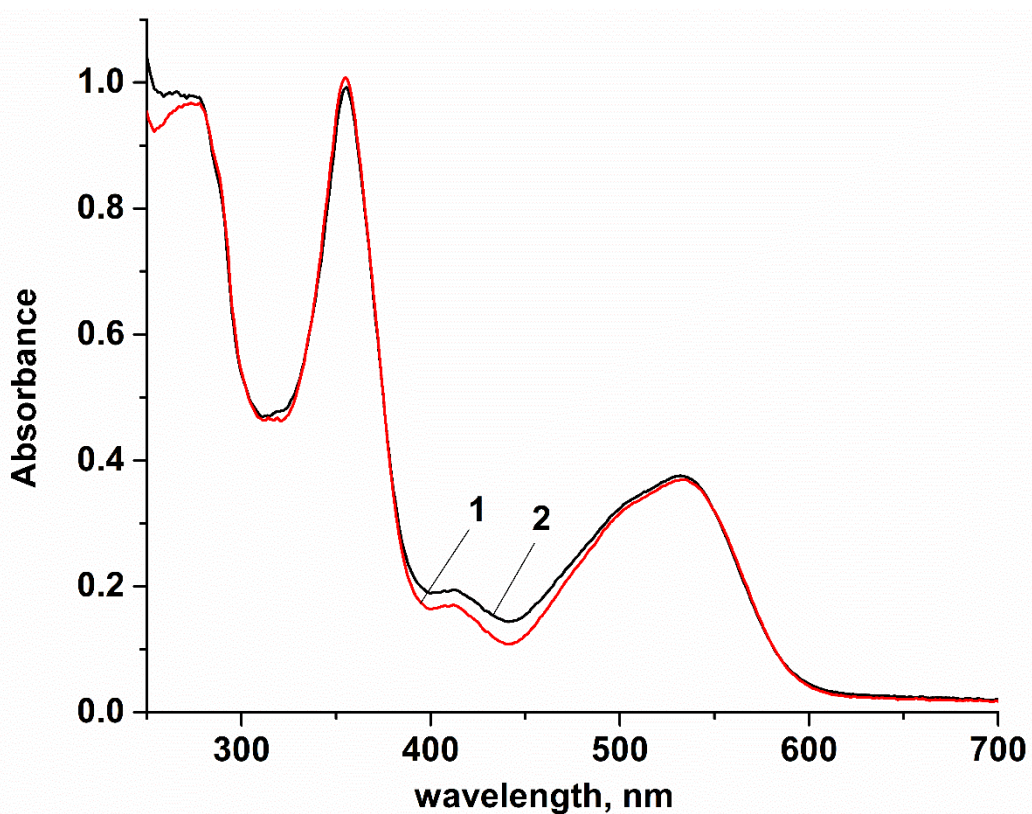


Figure S3. UV-vis spectra of  $\text{H}_2\text{OCbl}$  ( $4.5 \cdot 10^{-5}$  M) after mixing with  $\text{SCN}^-$  ( $1.0 \cdot 10^{-3}$  M; 1) and with  $\text{OSCN}^-$  (ca.  $6.0 \cdot 10^{-4}$  M)/ $\text{SCN}^-$  ( $1.0 \cdot 10^{-3}$  M) mixture prepared via basic hydrolysis of  $(\text{SCN})_2$  (2) at pH 6.5, 25.0 °C

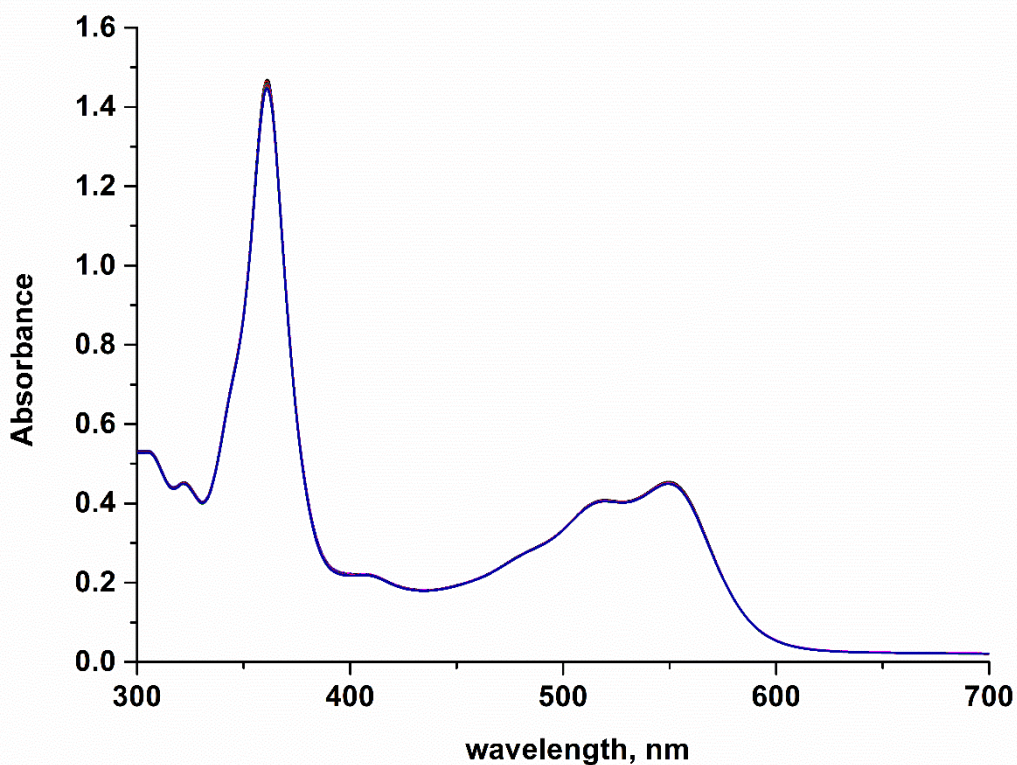


Figure S4. UV-vis spectra of the reaction between  $\text{CNCbl}$  ( $5.0 \cdot 10^{-5}$  M) and the mixture of LPO (ca. 0.6 U/ml),  $\text{SCN}^-$  (2.0 mM) and  $\text{H}_2\text{O}_2$  (1.0 mM) at pH 6.5 and 25.0 °C. Spectra were recorded for 1 h, time interval between spectra is 1 min

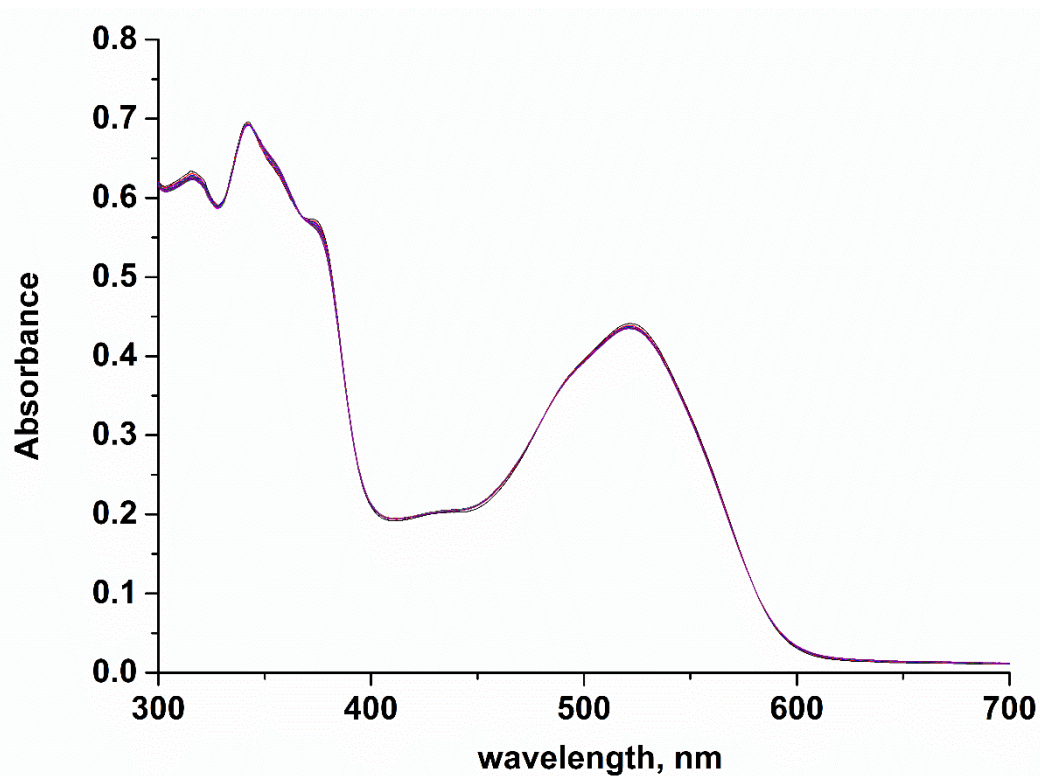


Figure S5. UV-vis spectra of the reaction between MeCbl ( $5.0 \cdot 10^{-5}$  M) and the mixture of LPO (ca. 0.6 U/ml),  $\text{SCN}^-$  (2.0 mM) and  $\text{H}_2\text{O}_2$  (1.0 mM) at pH 6.5 and 25.0 °C. Spectra were recorded for 1 h, time interval between spectra is 1 min

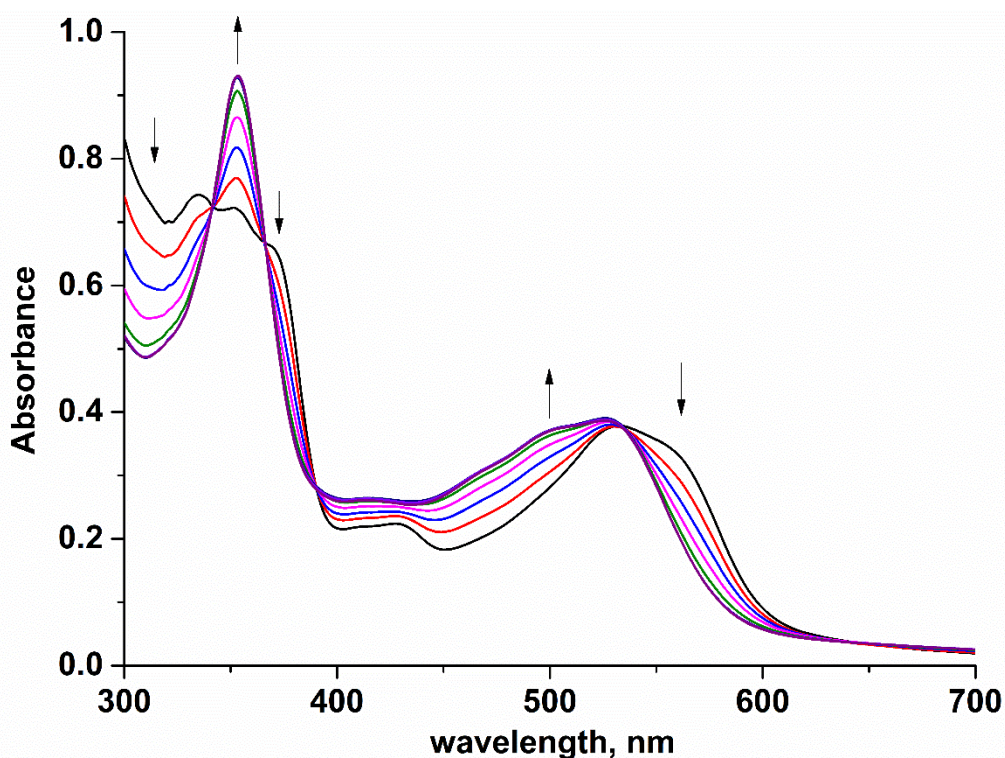


Figure S6. UV-vis spectra of the reaction between GSCbl ( $5.0 \cdot 10^{-5}$  M) and the mixture of LPO (ca. 0.6 U/ml),  $\text{SCN}^-$  (1.0 mM) and  $\text{H}_2\text{O}_2$  (1.0 mM) at pH 6.5 and 25.0 °C. Time interval between spectra is 1 min



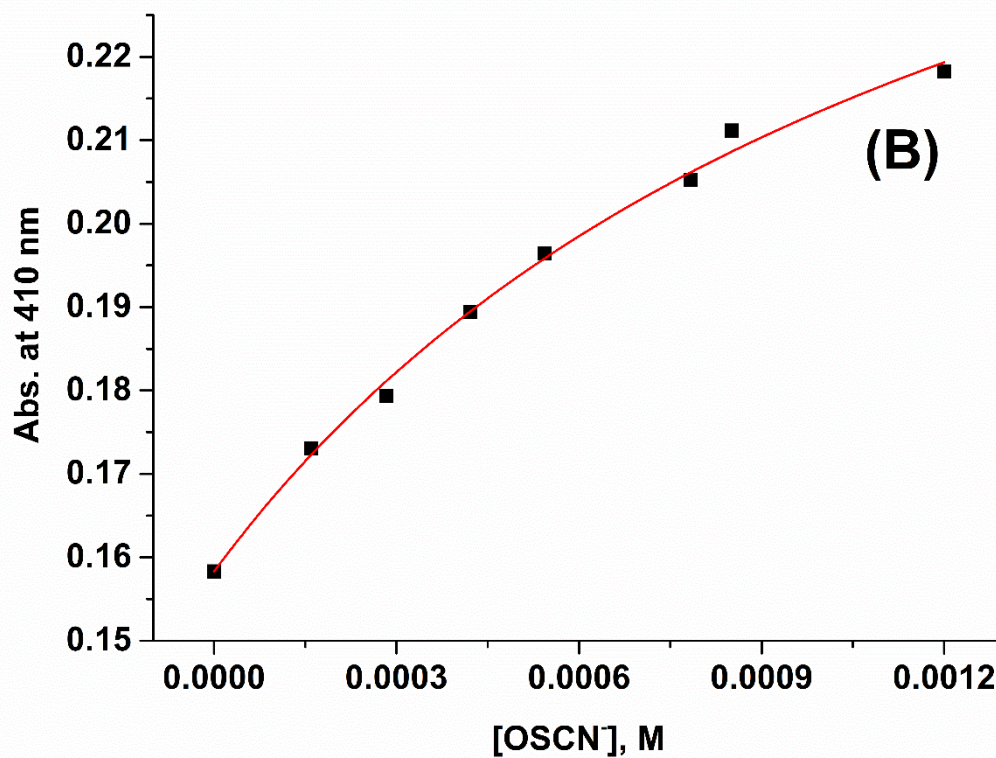
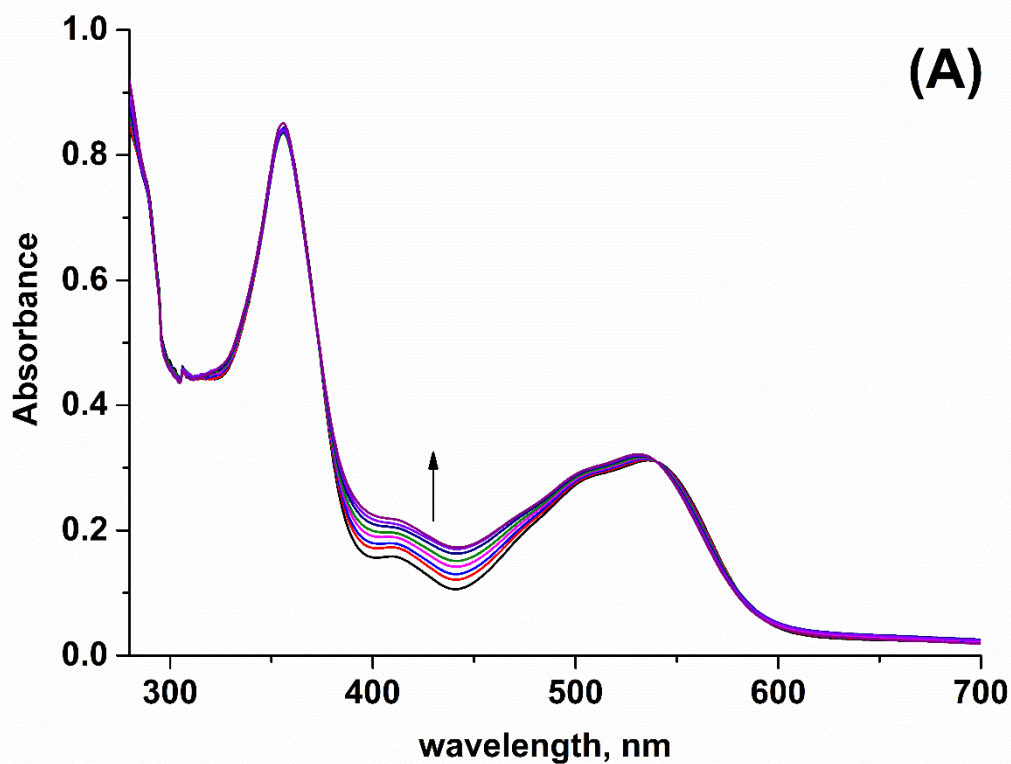


Figure S7. UV-vis spectra of mixtures containing thiocyanatocobalamin ( $5.0 \cdot 10^{-5}$  M) and different quantities of  $\text{OSCN}^-$  (B), and plot of absorbance at 410 nm versus  $[\text{OSCN}^-]$  (B) obtained at pH 6.5 (0.1 M phosphate buffer solution),  $25.0$  °C,  $[\text{SCN}^-] = 1.0 \cdot 10^{-2}$  M.  $\text{OSCN}^-$  was prepared by mixing  $\text{SCN}^-$  with various quantities of  $\text{H}_2\text{O}_2$  in the presence of LPO (ca. 0.6 U/ml). Using eq. (S1),  $K_{\text{obs.}} = (7.8 \pm 1.1) \cdot 10^2 \text{ M}^{-1}$  was determined

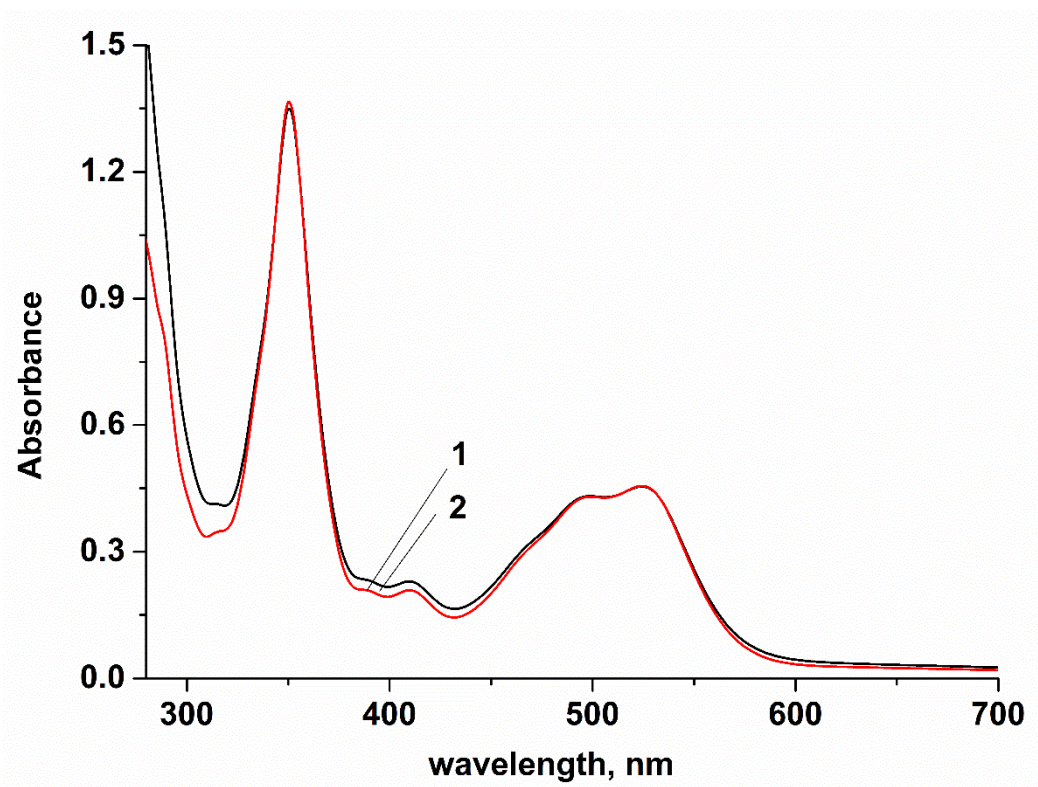


Figure S8. UV-vis spectra of  $\text{H}_2\text{OCbl}$  ( $5.0 \cdot 10^{-5}$  M; 1) and its mixture with selenomethionine ( $3.0 \cdot 10^{-2}$  M; 2) at pH 6.5 and 25.0 °C