Supporting Information

Label-free detection of fibrinogen based on fibrinogen-enhanced peroxidase activity of fibrinogen-hemin composite

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Fig. S1. UV–vis spectra of hemin (black), Fib (red) and the hemin-Fib (blue). Experimental conditions: hemin 25 µM, Fib 0.1 µM.
**Fig. S2.** CD spectra of hemin (red), Fib (blue) and the hemin-Fib (black).

Experimental conditions: hemin 25 µM, Fib 0.1 µM.

**Fig. S3.** TEM images of Fib (A), hemin (B), and hemin-Fib (C).
Fig. S4. SDS-polyacrylamide gel electrophoresis of Fib.
Fig. S5. Langmuir isotherms obtained from the Fib interactions with various concentrations of hemin at room temperature. (a) Non-linear regression between \(\Delta A\) and [Hemin], (b) linear regression between \([\text{Hemin}] / \Delta A\) and [Hemin]. Experimental conditions: Fib 50nM, ABTS 5 mM, \(\text{H}_2\text{O}_2\) 2.5 mM, 20 mM Tris-HCl (pH 8.0).

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\text{Hemin} + \text{Fib} \rightleftharpoons \text{Hemin-Fib} \tag{1}
\]

\[
K_b = \frac{[\text{Hemin-Fib}]}{[\text{Hemin}][\text{Fib}]} \tag{2}
\]

\[
\Delta A = \Delta A_{\text{max}} \cdot \frac{[\text{Hemin}]}{1 + [\text{Hemin}]K_b} \tag{3}
\]

\[
\frac{[\text{Hemin}]}{\Delta A} = \frac{1}{\Delta A_{\text{max}} K_b} + \frac{[\text{Hemin}]}{\Delta A_{\text{max}}} \tag{4}
\]
Fig. S6. The effect of catalytic activity on hemin. Experimental conditions: 100 μL of 5 mM ABTS, 100 μL of 2.5 mM H₂O₂, 100 μL of 20 mM Tris-HCl (pH 8.0), 7 min.

Fig. S7. The effect of catalytic activity on the interaction between hemin and Fib with difference pH. Experimental conditions: 100 μL of 5 mM ABTS, 100 μL of 2.5 mM H₂O₂, 100 μL of 20 mM Tris-HCl (pH 8.0), 50 μL of 25 μM hemin or hemin-Fib (Fib 50 nM, hemin 25 μM ), 7 min.
**Fig. S8.** The effect of catalytic activity on ABTS. Experimental conditions: 100 μL of 2.5 mM H₂O₂, 100 μL of 20 mM Tris-HCl (pH 8.0), 50 μL of 25 μM hemin or hemin-Fib (Fib 50 nM, hemin 25 μM), 7 min.

**Fig. S9.** The effect of catalytic activity on pH. Experimental conditions: 100 μL of 5 mM ABTS, 100 μL of 2.5 mM H₂O₂, 100 μL of 20 mM Tris-HCl, 50 μL of 25 μM hemin or hemin-Fib (Fib 50 nM, hemin 25 μM), 7 min.
**Fig. S10.** The effect of catalytic activity on the reaction time of ABTS-H$_2$O$_2$ system. Experimental conditions: 100 μL of 5 mM ABTS, 100 μL of 2.5 mM H$_2$O$_2$, 100 μL of 20 mM Tris-HCl (pH 8.0), 50 μL of 25 μM hemin or hemin-Fib (Fib 50 nM, hemin 25 μM), 7 min.