Supplementary information

Colorimetric detection of hydrogen peroxide and glucose by exploiting the peroxidase-like activity of papain

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**Fig. S1.** Steady-state kinetic assays of papain using Michaelis–Menten model, (A) at a constant concentration of H$_2$O$_2$ [5.0 mM] while TMB concentration was varied from 0.5 to 3.0 mM and (B) at a constant concentration of TMB [1.0 mM] while H$_2$O$_2$ concentration was varied from 0.25 to 2.0 mM. (C) Double-reciprocal plots of initial velocity versus TMB concentration and (D) Double-reciprocal plots of initial velocity versus H$_2$O$_2$ concentration.

**Fig. S2.** Effect of pH on the absorbance of the reaction system. (other conditions: 100 µL of 100 µg/mL papain; 1.0 mL of 5.0 mM TMB; 1.0 mL of 50µM H$_2$O$_2$; reaction temperature is 40°C; reaction time is 20 min.)
Fig. S3. Effect of temperature on the absorbance of the reaction system. (other conditions: 100 µL of 100 µg/mL papain; 1.0 mL of 5.0 mM TMB; 1.0 mL of 50 μM H₂O₂; pH=3.5; reaction time is 20 min.)

Fig. S4. Effect of reaction time on the absorbance of the reaction system. (other conditions: 100 µL of 100 µg/mL papain; 1.0 mL of 5.0 mM TMB; 1.0 mL of 50 µM H₂O₂; pH=3.5; reaction temperature is 40°C.)

Table S1. Comparison of kinetic parameters between papain and other catalysts

| Catalysts | [E] (M) | K_m (mM) | V_max (10^-8 M·s^-1) | K_cat (s^-1) | Reference |
|-----------|---------|----------|-----------------------|--------------|-----------|
|           |         | TMB      | H₂O₂                 | TMB          | H₂O₂      |           |
| HRP       | 2.27×10^{-11} | 0.15    | 0.61                 | 4.53         | 2.53      | 2.00×10^3 | 1.04×10^3 | [37]   |
| Ficin     | 4.20×10^{-8}  | 0.19    | 0.35                 | 4.69         | 3.42      | 1.12      | 0.81      | [37]   |
| Papain    | 4.27×10^{-8}  | 6.94    | 1.29                 | 12.03        | 2.11      | 2.82      | 0.49      | This work |

[E] is the enzyme concentration, K_m is the Michaelis constant, V_max is the maximum reaction rate, K_cat is the catalytic constant, where \( K_{cat} = \frac{V_{max}}{[E]} \)