Electronic supplementary information

Instrument-free quantitative detection of alkaline phosphatase using paper-based devices

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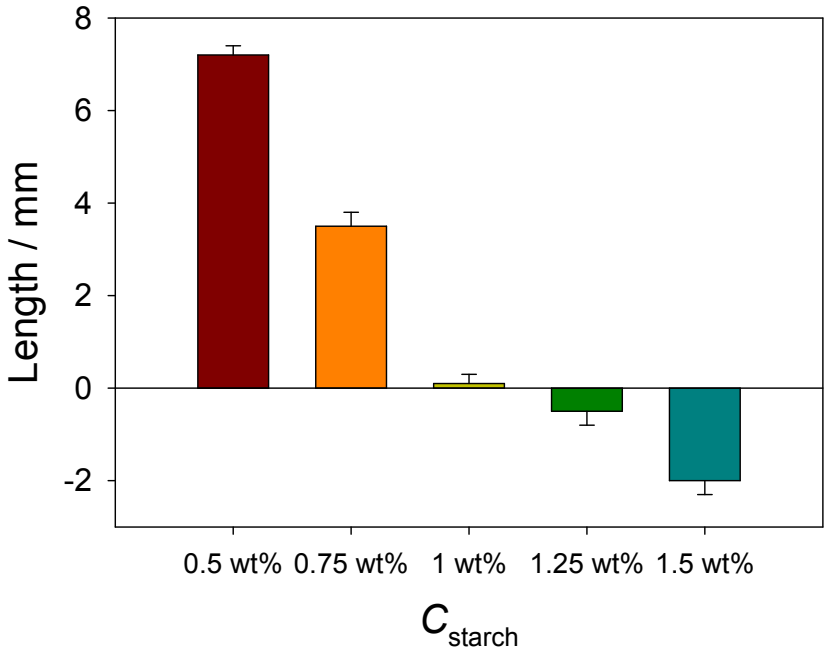

Fig. S1 Optimization of the starch concentration (C_{starch}). The smallest Cstarch that could completely block the flow of the red ink solution through the zone **b** in the used strip-like paper device, namely the 1 wt% was chosen in all the ALP assays.

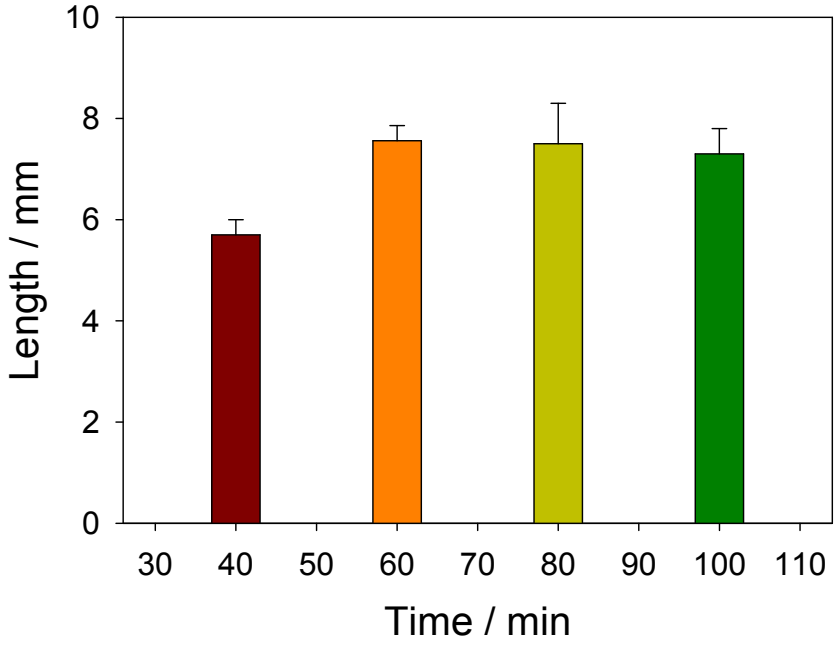

Fig. S2 Time optimization for the glucoamylase-catalysed hydrolysis of the starch. 60 min was chosen as the optimal hydrolysis time in all the ALP assays.

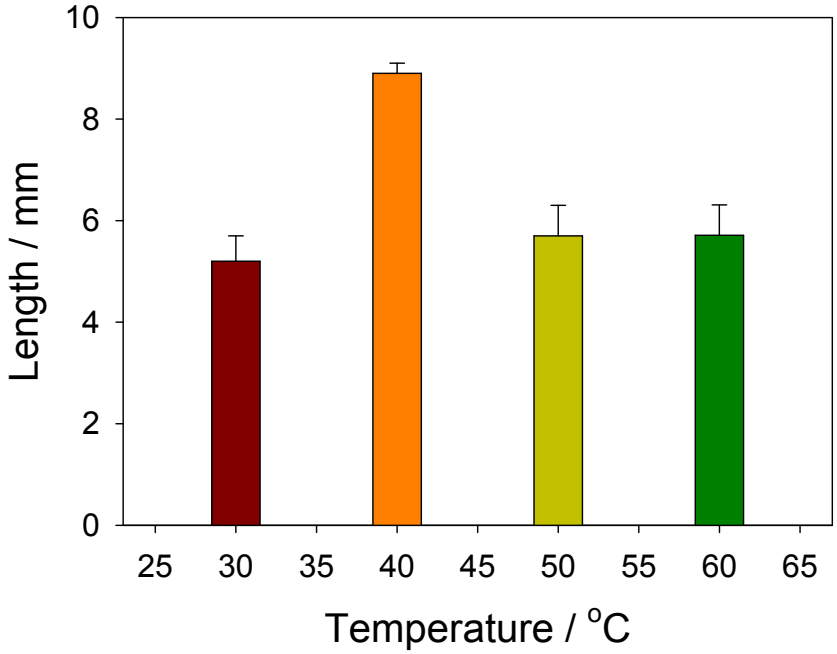

Fig. S3 Temperature optimization for the glucoamylase-catalysed hydrolysis of the starch. The temperature that could allow the glucoamylase to keep the highest activity to catalyse the hydrolysis of the starch to obtain the longest flow length of the red ink solution in the zone **c** in the used strip-like paper device, namely the 40 °C was chosen in all the ALP assays.

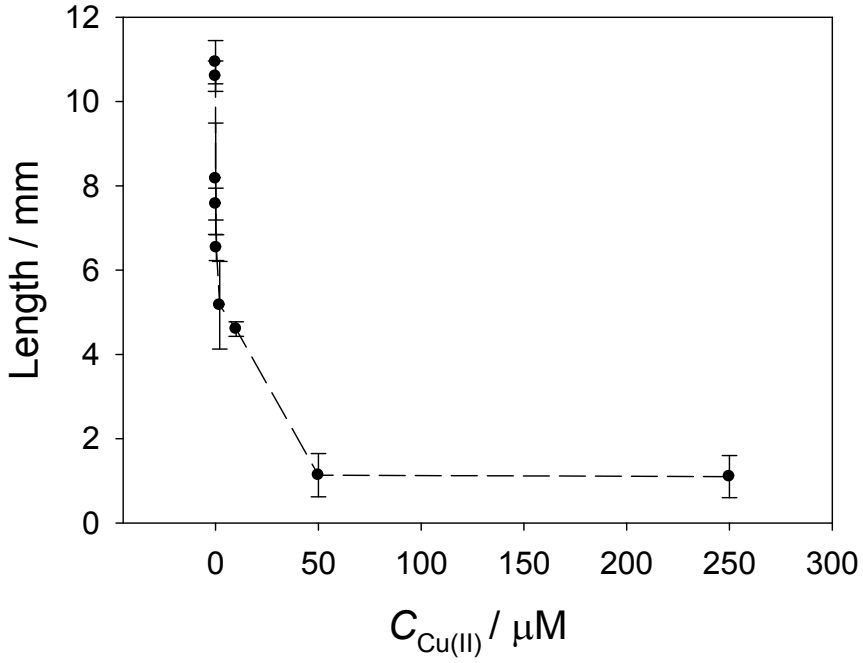

Fig. S4 Optimization of the concentration of $Cu(NO_3)_2$) ($C_{Cu(II)}$). The smallest $C_{Cu(II)}$ that could effectively inhibit the glucoamylase with a filled activity level to obtain the shortest flow length of the red ink solution in the zone **c** in the used strip-like paper device, namely the 50 μM was chosen in all the ALP assays.

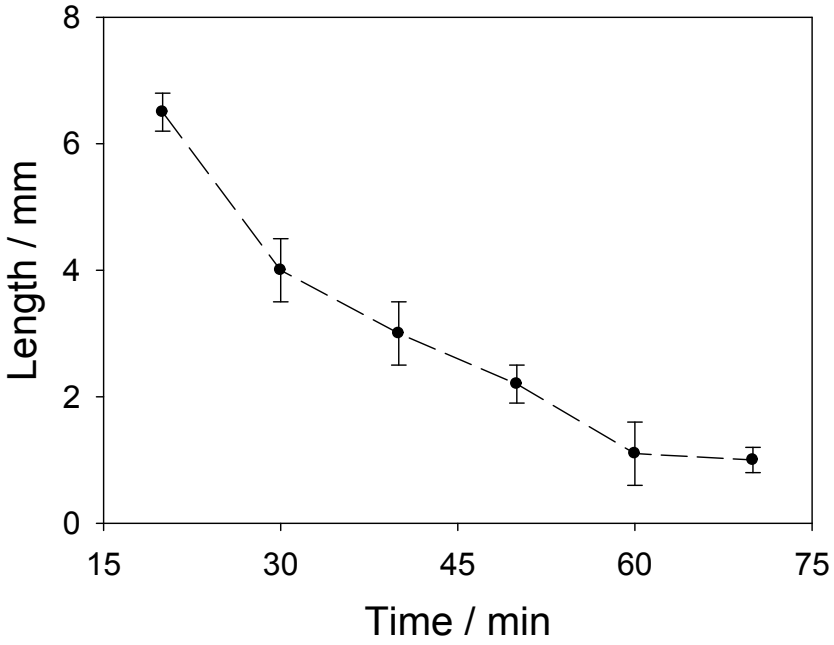

Fig. S5 Time optimization for the Cu^{2+}-glucoamylase inhibition reaction. The smallest time that could allow the Cu^{2+} to effectively inhibit the glucoamylase with a filled activity level to obtain the shortest flow length of the red ink solution in the zone **c** in the used strip-like paper device, namely the 60 min was chosen in all the ALP assays.

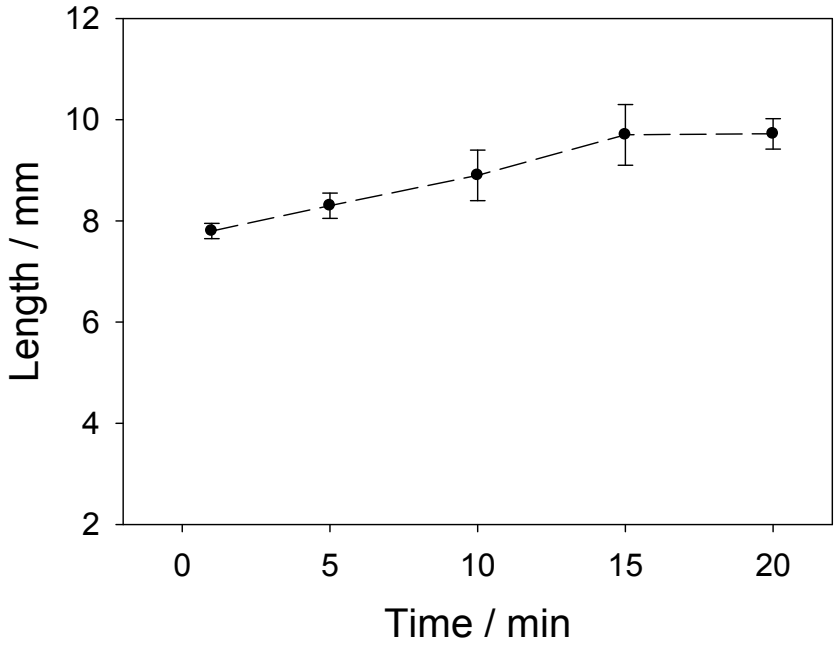

Fig. S6 Time optimization for the PPA-Cu^{2+} complexation. The smallest time that could finish the PPA-Cu^{2+} complexation reaction so that the glucoamylase could effectively keep its activity for catalysing the starch hydrolysis to obtain the longest flow length of the red ink solution in the zone **c** in the used strip-like paper device, namely the 15 min was chosen in all the ALP assays.

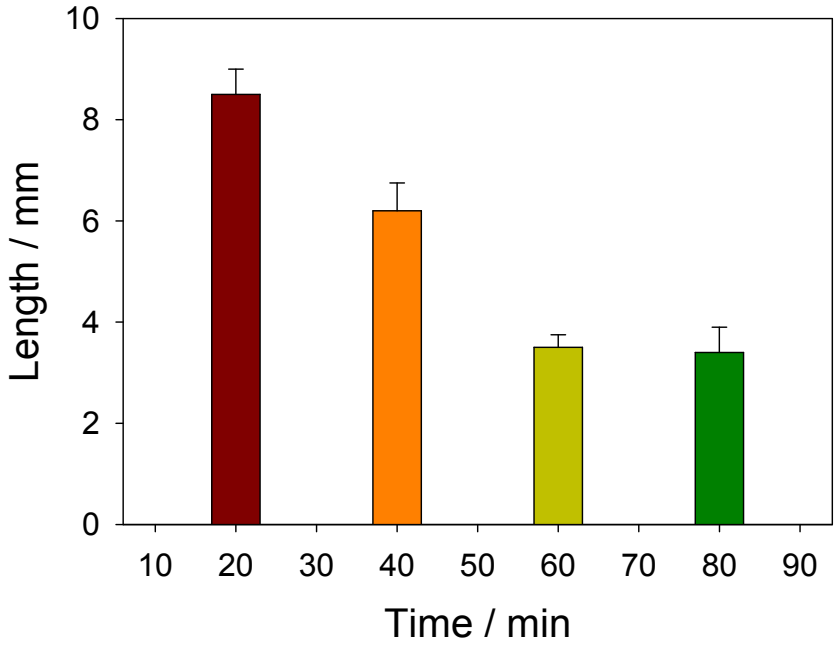

Fig. S7 Time optimization for the hydrolysis of PPA by ALP. The smallest time that could allow the ALP to effectively catalyse the PPA's hydrolysis so that the free Cu^{2+} could effectively inhibit the glucoamylase with a filled activity level to obtain the shortest flow length of the red ink solution in the zone **c** in the used strip-like paper device, namely the 60 min was chosen in all the ALP assays.

Table S1 Recovery of ALP in human serum samples

Sample	Found (U/mL)	Added (U/mL)	Estimated (U/mL)	Recovery （%）	RSD[a] (%, n=6)
1	0.000	0.075	0.073	97.3	6.6
2	0.000	0.625	0.578	92.5	5.9
3	0.000	1.250	1.380	110.4	5.5

[a] RSD indicates relative standard deviation.