Electronic Supplementary Information (ESI)

A portable thermal detection method based on target responsive hydrogel mediated self-heating of warm pad

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Materials and chemicals

Malathion and N,N,N′,N′-tetramethylethylenediamine (TEMED) were supplied by Sigma-Aldrich. Catalase was provided by Sangon Biotech Inc. (Shanghai, China). Acrylamide, NaCl, Tris (hydroxymethyl)methyl aminomethane (Tris), Ethylene diamine tetraacetic acid (EDTA), H_2O_2 (30%), and ammonium persulfate (APS) were acquired from Aladdin (Shanghai, China). HCl was purchased from Tianjin Zhiyuan Chemical Co. Ltd (Tianjin, China). Warm pad was acquired from Taobao (China, http://www.taobao. com). Ultrapure water (18.2 MΩ·cm) was obtained from a Milli-Q water purification system. Sequences used in this work were acquired by Sangon Biotech Inc. (Shanghai, China) and listed as follow:

Strand A (S-A): 5' -acrydite-TTT GAT TCA CAG ATG AGT- 3'

Strand B (S-B): 5' -acrydite-TTT AGG TGT GAC GGA TGA- 3'

Malathion aptamer: 5' -ACT CAT CTG TGA ATC TCA TCC GTC ACA CCT GCT CTT ATA CAC AAT TGT TTT TCT CTT AAC TTC TTG ACT GCT GGT GTT GGC TCC CGT AT- 3'

Fabrication of the catalase-based 3D DNA Hydrogel

The DNA hydrogel was constructed according to the previous report with minor modification.[1] 100 μM acrydite-modified S-A and S-B were dispersed in the stock solution containing 10 mM Tris-HCl buffer, 4% acrylamide, 200 mM NaCl, 1 mM EDTA (pH 7.4), respectively. The mixture of S-A and S-B was bubbled with nitrogen for 5 min to remove air. Then, 1.4% (v/v) of a freshly prepared solution consisting of initiator (0.05 g APS, 0.5 mL deionized water) and catalyst (25 μL TEMED, 0.5 mL deionized water) was added to the above mixture. Then, nitrogen

bubbling was carried out for 5 min to form polymer strand A (PS-A) and polymer strand B (PS-B). After that, to produce aptamer cross-linked hydrogel with catalase trapped inside, PS-A and PS-B were mixed with linker aptamer at a 1:1:1 molar ratio with the addition of catalase (300 U) followed by bubbling nitrogen for an additional 5 min. Finally, the polyacrylamide solution was incubated at 25 °C for 20 min to form a catalase-based 3D DNA hydrogel.

Design of the Assay Process

10 μL target malathion with different concentrations was incubated with the above-mentioned 3D DNA hydrogel for 1 h to release the corresponding catalase, which was marked as a gas-generating bottle. Following that, the infusion tube needle was quickly inserted into the thermo-generating system with the thermometer probe beforehand inserted and quickly sealed with tape. And the gas-generating system was connected with a thermo-generating bottle through a catheter, then sealed and vacuumed. Finally, 0.4 mL 15% H_2O_2 was quickly injected into the gas-generating bottle, which would be converted to O_2. The O_2 causes the warming pad powder to heat up, and the temperature variation is recorded with a thermometer within 5 minutes.
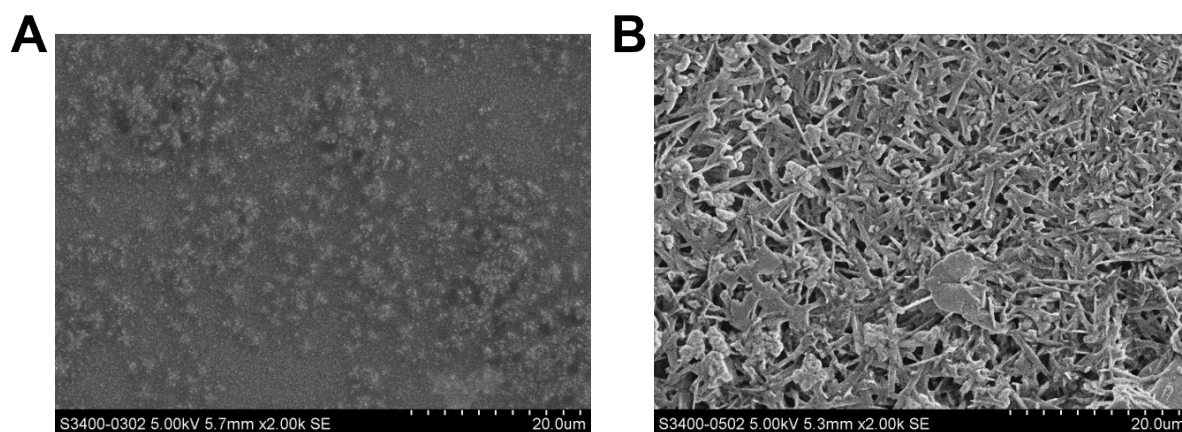
Characterization of DNA Hydrogel

Fig. S1 SEM images of hydrogel without (A) or with (B) malathion stimuli responsive.

Real Sample Analysis of Malathion in Red Wine

Firstly, wine sediment was removed by filtering the red wine sample. The filtered red wine sample was diluted 20-fold and adjusted to pH 7.4 for subsequent malathion analysis. Various concentrations of malathion were added into the red wine samples to obtain standard solutions (0.001, 0.1, 0.5, 10 ng mL^{-1}). The subsequent thermal detection was similar to the above assay process.

Optimization of assay conditions

Certainly, in order to achieve an optimum state for malathion assay, some corresponding experimental parameters such as the concentration of H_2O_2, the DNA cross-link density, and the reaction time of target and hydrogel were investigated. As shown in Figure S2A, the temperature variation initially increased with the increasing H_2O_2 concentration and then decreased, which might be ascribed to the inhibition of high concentration of H_2O_2 to the catalytic efficiency of catalase [2]. The detectable signal achieved the optimum state at 20% H_2O_2. Meanwhile, the result of drainage gas collection method was in agreement with the thermal experiment. Thus, 20% H_2O_2 was applied as the optimized concentration.

In addition, the stability of DNA hydrogel is strongly related to the sensitivity of the system. Thus, the parameter of the DNA cross-link density is optimized. As seen in Figure 2B, different DNA cross-linking density hydrogel with Au NP or catalase trapped inside were synthesized and detected by naked-eyes and thermometer. The 0.01 mM hydrogel has a great response to malathion but without encapsulating stability. As for the 0.05 mM and 0.1 mM hydrogels, they have a similar response, but 0.1 mM hydrogel has better stability. The 0.2 mM hydrogel, a slower reaction rate was acquired. As shown above, low-density cross-linking hydrogel probably dissolves much faster than high-density hydrogel, but it was not stable enough. However, high concentration hydrogel would be slower for the phase transition from hydrogel to a solution. So, 0.1 mM DNA was used for the preparation of DNA hydrogel.

The incubation time of target with the aptamer cross-linked hydrogel was also investigated. As displayed in Figure 2C, the temperature variation of the assay increased with the increasing reaction time and tended to be stabilized after 60 min. Hence, 60 min was chosen for the measurement in this study.
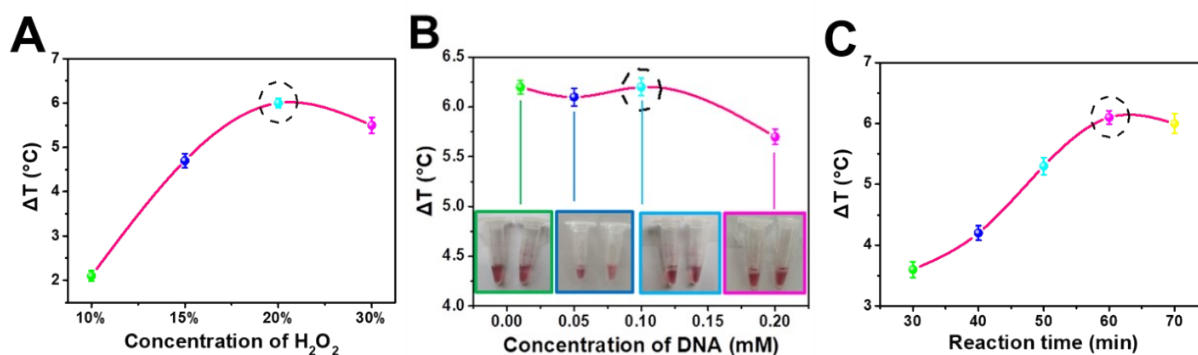

Fig. S2 The effects of (A) concentration of H_2O_2; (B) concentration of DNA; (C) reaction time between malathion and hydrogel. (10 ng mL^{-1} malathion used in this case)

Compared with other reports

Table S1 Comparison of assay performance between the developed sensing platform and other strategies.

Method	LOD	Linear range	Reference
Electrochemical Sensor	0.5 ng mL^{-1}	0.001-0.5 μg mL^{-1}	(Ding et al.2014)[3]
Electrochemical Sensor	0.172 ng mL^{-1}	0.025-0.85 ng mL^{-1}	(Shamgsumova et al.2018)[4]
Fluorescence Sensor	1.42 nM (0.4691 ng mL^{-1})	0.01-1 μM (3.304-330.36 ng mL^{-1})	(Chen et al.2020)[5]
Resonance Rayleigh Scattering Sensor	5.24 ng mL^{-1}	20-100 ng mL^{-1}	(Lai et al.2020)[6]
Colorimetric Sensor	0.5 pM (0.165 pg mL^{-1})	0.01–0.75 nM (3.3 pg mL^{-1}-0.248 ng mL^{-1})	(Bala et al.2018)[7]
Thermal Sensor	0.032pg mL^{-1}	0.0001-10 ng mL^{-1}	This work

Real Sample Analysis

To evaluate the practicability of the proposed thermal assay in real samples, various concentrations of malathion were added into diluted red wine as spiked samples. The recoveries at 0.1, 10, 100, and 1000 ng ml-1 of target were corresponding estimated as 100.4%, 99.9%, 100.1%, and 101.1%. The detection relative standard deviation (RSD) was less than 4.0%，demonstrating acceptable reliability of the proposed method in the complex samples.

Table S2 Recovery of Mal in spiked milk samples (n = 3)

Sample	Added (ng ml^{-1})	RSD (%)	Recovery (%)
1	0.1	2.5	100.4
2	10	4.0	99.9

3	100	3.4	100.1
4	1000	2.4	101.1

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