Electronic Supplementary Information

Time-Dependent Nanogel Aggregation for Naked-Eye Assays of α-Amylase Activity

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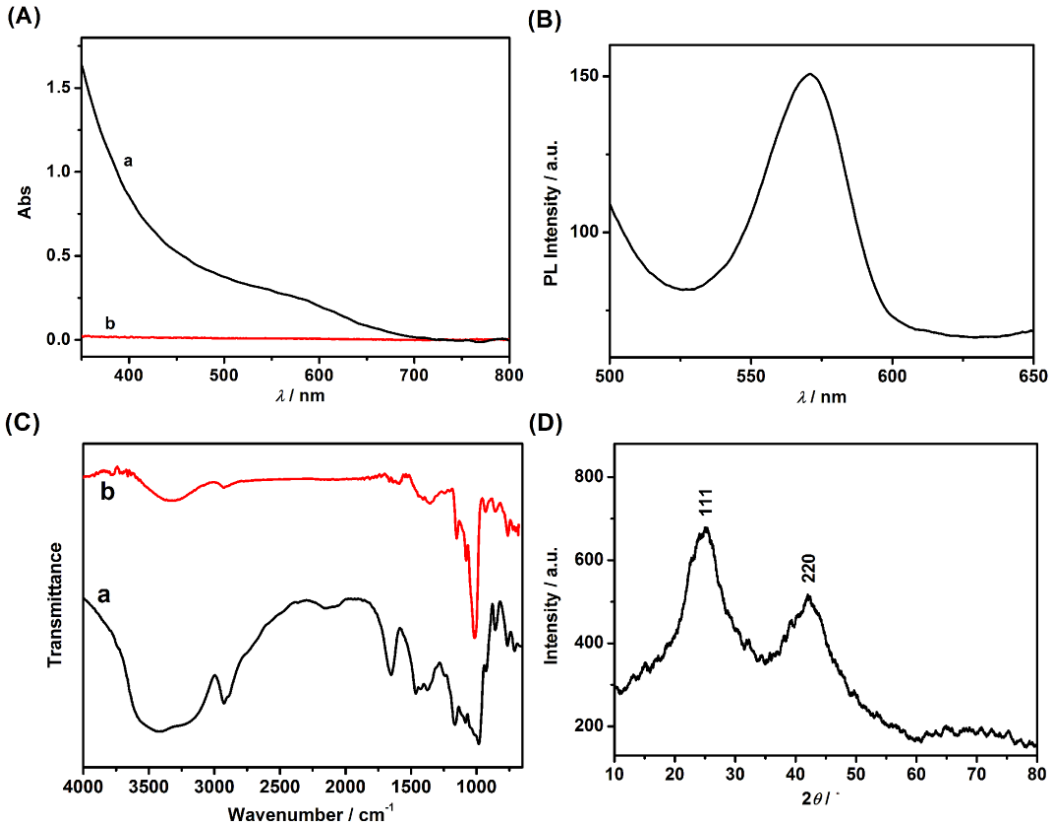

Figure S1 (A) The UV-vis spectra before (a) and after (b) the α-amylase induced aggregation of the CdTe/St nanoparticles; (B) The photoluminescent spectrum of the CdTe/St nanoparticles (with an excitation wavelength of 350 nm); (C) The FT-IR spectrum of the CdTe/St nanoparticles (b) and starch (a); (D) The X-ray diffraction (XRD) pattern of the CdTe/St nanoparticles.

Table S1. Aggregation time of the nanogels in PB judged by 5 independent volunteers (V1~V5).

Activity / U mL^{-1}	V1 / s	V2 / s	V3 / s	V4 / s	V5 / s	Average / s	CV / %
5.0	40	37	41	40	39	39.4±1.5	3.8
1.25	79	78	76	81	82	79.2±2.4	3.0
0.25	221	207	225	216	227	219.2±8.0	3.6

Deduction of the reciprocal relationship between the enzyme activity and the aggregation time

For the typical enzymatic catalytic process, the classical Michaelis-Menton kinetics is used to fix the model:

$$\mathrm{E} + \mathrm{CdTe/St} \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} \mathrm{E\text{-}CdTe/St} \xrightarrow{k_0} \mathrm{E} + \mathrm{P}$$

First we denote:

$[E]_0$, initial amylase concentration;

$[E]$, amylase concentration at certain time;

$[CdTe/St]_0$, initial substrate (CdTe/St) concentration;

$[CdTe/St]$, substrate concentration at certain time;

$[E\text{-}CdTe/St]$, the concentration of the enzyme-substrate complex;

$[P]$, the concentration of the productes.

Define the kinetic constant for the formation and dissociation of the E-CdTe/St and generation of the product is k_1, k_{-1}, and k_0, respectively. Then

$$-\frac{d[CdTe/St]}{dt} = k_1[E][CdTe/St] - k_{-1}[E\text{-}CdTe/St] \quad (1)$$

For an equilibrium state,

$$-\frac{d[E\text{-}CdTe/St]}{dt} = k_1[E][CdTe/St] - (k_{-1} + k_0)[E\text{-}CdTe/St] = 0 \quad (2)$$

Define $K_M=(k_{-1}+k_0)/k_1$, then $$[E\text{-}CdTe/St] = \frac{[E][CdTe/St]}{K_M} \quad (3)$$

While $[E]_0 = [E]+[E\text{-}CdTe/St]$ (4), the following equation (5) and (6) can be obtained:

$$[E] = \frac{K_M[E]_0}{K_M + [CdTe/St]} \quad (5)$$

$$[E\text{-}CdTe/St] = \frac{[E]_0[CdTe/St]}{K_M + [CdTe/St]} \quad (6)$$

Put Eqn. (5) and (6) in Eqn. (1),

$$-\frac{d[CdTe/St]}{dt} = k_1 \frac{K_M[E]_0[CdTe/St]}{K_M + [CdTe/St]} - k_{-1} \frac{[E]_0[CdTe/St]}{K_M + [CdTe/St]} = k_0[E]_0 \frac{[CdTe/St]}{K_M + [CdTe/St]}$$

(7)

Using calculus, $$-\int_{[CdTe/St]_0}^{[CdTe/St]} d[CdTe/St]\left(\frac{K_M}{[CdTe/St]} + 1\right) = \int_0^t k_0[E]_0\, dt$$

$$\text{thus} \quad k_0[E]_0 t = ([CdTe/St]_0 - [CdTe/St]) + K_M \ln\frac{[CdTe/St]_0}{[CdTe/St]} \quad (8)$$

According to the law of conservation of mass, suppose the substance (CdTe/St) in the enzymatic reaction turns to products (P) by *n*-fold.

$$\begin{aligned} \frac{[P]}{n} &= [CdTe/St]_0 - [CdTe/St] - [E\text{-}CdTe/St] \\ &= [CdTe/St]_0 - [CdTe/St]\left(1 + \frac{[E]_0}{K_M + [CdTe/St]}\right) \end{aligned} \quad (9)$$

Since $[E]_0 \ll [CdTe/St]$, (9) can be simplified as:

$$\frac{[P]}{n} \approx [CdTe/St]_0 - [CdTe/St] \quad (10)$$

From Eqn. 10, the $[CdTe/St]_{agg}$ is fixed when the concentration of the product reaches a critical value ($[P]_{agg}$) to induce the aggregation of the nanoparticles. Then the right hand side in Eqn. 8 is a constant and we can conclude that

$$t_{agg} \propto 1/[E]_0$$

Standard protocol for assays of α-Amylase activity [S1]

Firstly, 12.0 g of sodium potassium tartrate tetrahydrate was dissolved in 8.0 mL of 2 M NaOH to prepare the sodium potassium tartrate solution. This solution was added to 20 mL of 96 mM 3, 5-dinitrosalicylic acid solution and then dilute to 40 mL with deionized water to obtain the color reagent solution (Fig S2A), which is stored in an amber bottle at room temperature and can be stable for 6 months. The maltose standard solution of 0.2% (w/v) was prepared by 20 mg maltose in 10 mL deionized water. The standard (Std) experiments are performed as steps in Table S2. A

calibration curve between $\Delta A_{540\ nm}$ [i.e., $A_{540\ nm}$ (Std) - $A_{540\ nm}$ (Std Blank)] and the mass of the maltose (mg) could be plotted (Fig S2B inset).

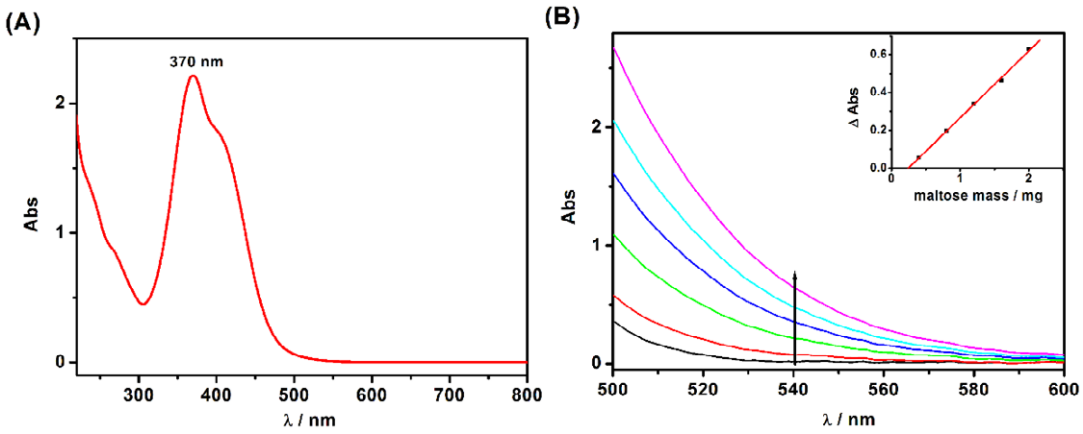

Figure S2 (A) The UV-vis spectrum of the color reagent solution; (B) The spectroscopic curves in the presence of different volume of maltose solution. Inset: the calibration curves by $\Delta Abs_{540\ nm}$ *vs* mass of maltose.

Table S2 Standard protocols for the achievement of the calibration curve

	Std Blank / mL	Std 1 / mL	Std 2 / mL	Std 3 / mL	Std 4 / mL	Std 5 / mL
maltose solution	0	0.20	0.40	0.60	0.80	1.00
deionized water	2.00	1.80	1.60	1.40	1.20	1.00
color reagent solution	1.00	1.00	1.00	1.00	1.00	1.00
Place in a boiling water bath for exactly 15 minutes, then cool on ice to room temperature						
deionized Water	9.00	9.00	9.00	9.00	9.00	9.00
Mix by inversion and record the A_{540nm} for the Standards and Standard Blank using a spectrophotometer						

To determine α-Amylase activity, the enzyme solution was prepared by dissolution of the commercial α-Amylase reagent in deionized water at room temperature immediately before use. The saliva samples are collected as the standard procedures. The substrate, i.e., 1.0% (w/v) soluble starch solution was prepared by 0.25 g starch in 25 mL of 20 mM PBS (pH 6.8, containing 6.7 mM NaCl). The solubilization is facilitated by heating the solution to boil and maintain the solution at

this temperature for 15 minutes. The assays for the samples and blank were operated according to the procedures listed in Table S3. Then $\Delta A_{540\ nm}$ = [$A_{540\ nm}$ (Test) - $A_{540\ nm}$ (Blank)] can be calculated and placed in the calibration curve (Fig S2B inset) to obtain the mass of maltose (mg). According to the definition, 1 mg maltose means 1 U. The enzyme activity can thus be determined.

Table S3 Standard protocols for assays of amylase activity

	Sample / mL	Blank / mL
1.0% (w/v) soluble starch solution	1.00	1.00
Mix by swirling and equilibrate to 20 °C		
Add: enzyme solution	1.00	0.00
Mix by swirling and incubate for exactly 3.0 min at 20 °C		
Add: color reagent solution	1.00	1.00
Add: enzyme solution	0.00	1.00
Place in a boiling water bath for exactly 15 mins, then cool on ice to room temperature		
Add: deionized water	9.00	9.00
Mix by inversion and record the A_{540nm} for both the Test and Blank		

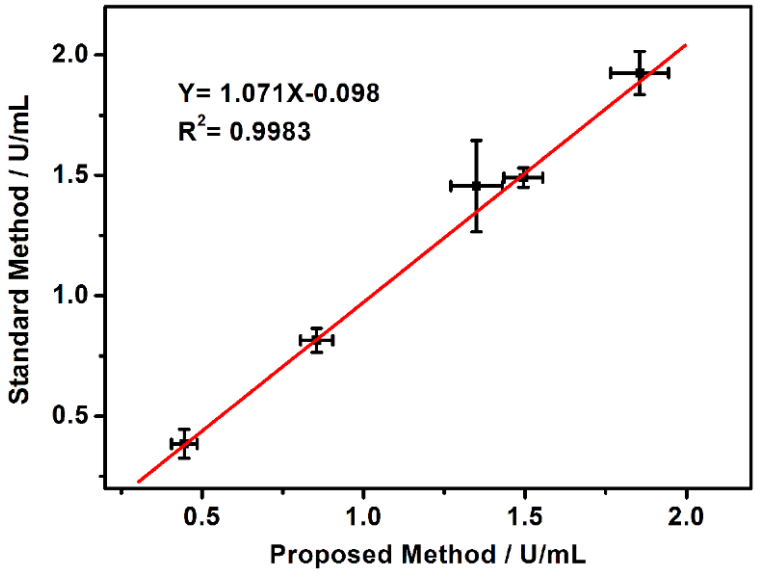

Figure S3 The correlation results between the proposed and the standard protocol for the assays of the α-amylase activity.

Table S4. Determination of α-amylase spiked in 25-fold diluted serum samples by aggregation time of CdTe/St nanogels during 5 Days (D1~D5)*

Activity / U mL^{-1}	D1 / s	D2 / s	D3 / s	D4 / s	D5 / s	Interday / s
5.0	53.7±1.2	56.0±1.0	55.7±1.5	54.3±3.1	55.3±2.5	55.0±1.0
CV (%)	2.2	1.8	2.7	5.7	4.5	1.8
2.5	77.3±3.2	79.7±2.5	79.0±2.0	75.3±2.5	79.0±2.6	78±1.8
CV (%)	4.1	3.1	2.5	3.3	3.3	2.3

***** The detection solution (100 μL) contains 25 μL PB (pH 6.8) of 200 mM, 31 μL deionized water, 5 μL 25-fold diluted serum sample spiked with 1 μL commercial α-amylase, and 38 μL CdTe/St of 1.6 mg mL^{-1}. The final α-amylase activity is 5 or 2.5 U mL^{-1}. Each point is measured in triplicate.

Table S5. Determination of α-amylase spiked in 25-fold diluted serum samples by using 3 parallel batches (S1~S3) of CdTe/St nanogels*

Activity / U mL^{-1}	S1 / s	S2/ s	S3 / s	Interbatch / s
5.0	53.7±1.2	55.7±2.1	56.3±3.1	55.2±1.4
%CV	2.2	2.8	5.5	2.5
2.5	77.3±3.2	80.7±2.5	78.7±2.1	78.9±1.7
%CV	4.1	3.1	2.7	2.2

***** Each point is measured in triplicate at Day 1.

Reference

[S1] Bernfeld, P. Methods in Enzymology 1955, 1, 149-158.