Supporting Information

Au@Pt nanodendrites enhanced multimodal enzyme-linked immunosorbent assay

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Materials and instruments

Carbon dots (CDs) were kindly provided by Prof. Yang at Jilin University, which was prepared by his previous work.[1] Chloroauric acid ($HAuCl_4$), pluronic F127, potassium hexachloroplatinate (K_2PtCl_6), ascorbic acid and bovine serum albumin (BSA) were purchased from Aladdin-industrial-corporation. O-phenylenediamine (OPD) and hydrogen peroxide were obtained from Shanghai Macklin Biochemical Co. Ltd. (Shanghai, China). Cardiac troponin I (cTnI) and cTnI antibodies were purchased from Shanghai Linc-Bio Science Co. Ltd. (Shanghai, China). Phosphate-buffered saline (PBS) was mixed by 0.05 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride.

Photothermal conversion efficiency was confirmed by an ADR-1860 laser from Changchun Laser Optoelectronics Technology Co. Ltd. The temperature was recorded by a pen-style digital thermometer (model number EW300) with a detection range of -50 to +300^{o}C was obtained from Zhengzhou Boyang Instrumentation Co. Ltd. The UV-vis absorption spectrum and fluorescent spectra were recorded by an automatic microplate reader (TECAN SPARK 20M, Switzerland). The transmission electron microscopy (TEM) images were collected on a Philips CM200 UT microscope (Field Emission Instruments, USA). Scanning electron microscope (SEM) images were obtained using field emission SEM (ZEISS, Germany) which operated at a 15.00 kV high voltage. The X-ray diffraction (XRD) was conducted from 20^0 to 85^0 (Bruker AXS, Germany).

Preparation of Au@Pt nanodendrites and bioconjugated with antibodies

20 mg of Pluronic F127 was ultrasonically dissolved in 1.8 mL of K_2PtCl_6(20 mM) and 0.2 mL of $HAuCl_4$ (1%wt) and 44 μL of hydrochloric acid (6 M). After adding 2.0 mL of 100 mM ascorbic acid as a reducing agent, the mixture was continuously sonicated. As the reaction proceeded, the solution color changed to pink. The mixture was continuously sonicated in a water bath for 4 h. The Au@Pt nanodendrites was collected and washed with acetone and water in consecutive washing/centrifugation cycles for five times and then dried at room temperature.

1 mg of Au@Pt nanodendrites were mixed with 100 μg/mL of Ab_2 (1 mL) at 37 ℃ for incubation with 60 minutes. Next, the mixture Au@Pt-Ab_2 was washed for 3 times to remove unbonded antibodies. Finally, Au@Pt-Ab_2 was re-dispersed in 1 mL of PBS (pH 7.4) and stored at 4 ℃ until used.

Calculation of photothermal conversion efficiency for as-prepared Au@Pt nanodendrites

According to previous work, [2,3] the total energy balance towards this system can be expressed as equation 1:

$$\sum_t m_i C_{p,t} \frac{dT}{dt} = Q_{Au@Pt} + Q_{Dis} - Q_{Suur} \quad (1)$$

Where m and C_p are the mass and heat capacity of water, respectively, T is the temperature of the solution, $Q_{Au@Pt}$ is the energy inputted by Au@Pt nanodendrites, Q_{Dis} is the baseline energy inputted by the sample cell, and Q_{Surr} is heat conduction away from system surface by air.

The laser-induced source term, $Q_{Au@Pt}$, represents heat dissipated by electron-phonon

relaxation of the plasmons on the Au@Pt nanodendrites surface under the irradiation of 808 nm laser:

$$Q_{Au@Pt} = I\left(1 - 10^{-A_{808}}\right)\eta \quad (2)$$

Where I is incident laser power, η is the conversion efficiency from incident laser energy to thermal energy, and A_{808} is the absorbance of Au@Pt nanodendrites at a wavelength of 808 nm (**Figure S1a**). In addition, source term, Q_{Dis}, expresses heat dissipated from light absorbed by the quartz sample cell itself. Furthermore, Q_{Surr} is linear with temperature for the outgoing thermal energy, as given by equation 3:

$$Q_{Suur} = hs\left(T - T_{Suur}\right) \quad (3)$$

Where h is heat transfer coefficient, S is the surface area of the container, and T_{Surr} is the ambient temperature of the surroundings.

Once the laser power is defined, the heat input ($Q_{Au@Pt}$ + Q_{Dis}) will be finite. Since the heat output (Q_{Surr}) is increased along with the increase of the temperature according to the equation 3, the system temperature will rise to a maximum when the heat input is equal to heat output:

$$Q_{Au@Pt} + Q_{Dis} = Q_{Surr-Max} = hs\left(T_{max} - T_{Surr}\right) \quad (4)$$

Where the $Q_{Surr\text{-}Max}$ is heat conduction away from the system surface by air when the sample cell reaches the equilibrium temperature, and T_{max} is the equilibrium temperature. The 808 nm laser heat conversion efficiency (η) can be determined by substituting equation 2 for $Q_{Au@Pt}$ into equation 5 and rearranging to get

$$\eta = \frac{hs\left(T_{Max} - T_{Surr}\right) - Q_{Dis}}{I\left(1 - 10^{-A_{808}}\right)} \quad (5)$$

Where Q_{Dis} was measured independently, the (T_{max}-T_{Surr}) was 29.6 °C according to Figure 2a, I is 0.71 mW/cm^2, A_{808} is the absorbance (0.57) of Au@Pt nanodendrites at 808 nm (**Figure S1a**). Thus, only the hS remains unknown for calculating η.

In order to get the hS, a dimensionless driving force temperature, θ is introduced using the maximum system temperature, T_{max}

$$\theta = \frac{T - T_{Surr}}{T_{Max} - T_{Surr}} \quad (6)$$

and a sample system time constant τ_s

$$\tau_s = \frac{\sum_i m_i C_{p,i}}{hs} \quad (7)$$

which is substituted into the equation. 1 and rearranged to yield

$$\frac{d\theta}{dx} = \frac{1}{\tau_s}\left[\frac{Q_{Au@Pt} + Q_{Dis}}{hs(T_{Max} - T_{Surr})} - \theta\right] \quad (8)$$

At the cooling stage of the aqueous dispersion of the Au@Pt nanodendrites, the light source was shut off, the $Q_{Au@Pt}$ +Q_{Dis} = 0, reducing the equation 9

$$dt = -\tau s\frac{d\theta}{\theta} \quad (9)$$

and integrating, giving the expression

$$t = -\tau_s ln\theta \quad (10)$$

Therefore, time constant for heat transfer from the system is determined to be τ_s =477.9 by applying the linear time data from the cooling period vs negative natural logarithm of driving force temperature (**Figure S1c and d**). In addition, the m is 1.0 g and the C is 4.2 J/g. Thus, according to Eq. 7, the hS is deduced to be 8.8 mW/°C. Substituting 8.8 mW/°C of the hS into Eq. 5, the 808 nm laser heat conversion

efficiency (η) of Au@Pt nanodendrites can be calculated to be 45.1%.

Fabrication of immunosensor

100 μL of Ab_1 with the amount of 1 μg/mL diluted in PBS (pH 7.4) was incubated with 96-well plates for 12 h at 4 ℃. Next, these plates were washed with PBS (pH 7.4) for 3 times to remove unbonded antibodies. Afterward, 200 μL of BSA (1%) diluted in PBS (pH 7.4) was added and incubated for 30 minutes at 37 ℃ to block nonspecific active sites and washed for 3 times to remove excess BSA. Next, 100 μL of different concentration of cTnI was added into each well and incubated for 30 minutes at 37 ℃. After that, each well was washed with PBS (pH 7.4) for 3 times to remove unbonded antigen and 100 μL of Au@Pt-Ab_2 (0.5 mg/mL) was subsequently added into each well and incubated for 60 minutes at 37 ℃. Each well was washed with PBS (pH 7.4) for 3 times to remove unbonded Au@Pt-Ab_2.

Photothermal immunoassay of cTnI

Firstly, the immunosensor with different concentration of cTnI was irradiated with an 808 nm laser (0.71 W/cm^{-2}) for 10 minutes. Next, the temperature of immunosensor was recorded by a pen-style digital thermometer.

Colorimetric and ratiometric fluorescent immunoassay of cTnI

Firstly, 10 μL of CDs (0.02 mg/mL) was added into each well with different concentration of cTnI rapidly and 200 μL of OPD (15 mM) diluted in HAC-NaAC (pH 4.0) was incubated for 7 minutes at 37 ℃. Next, the UV-vis absorption spectrum and fluorescence spectra were recorded by an automatic microplate reader.

Analysis in clinical serum sample

The clinical serum samples provided by Department of Urology, Renji Hospital of Shanghai Jiao Tong University Medicine School were diluted 100 times with PBS (pH = 7.4) and did not need additional pretreatment procedures. After that, the obtained samples were detected using our method.

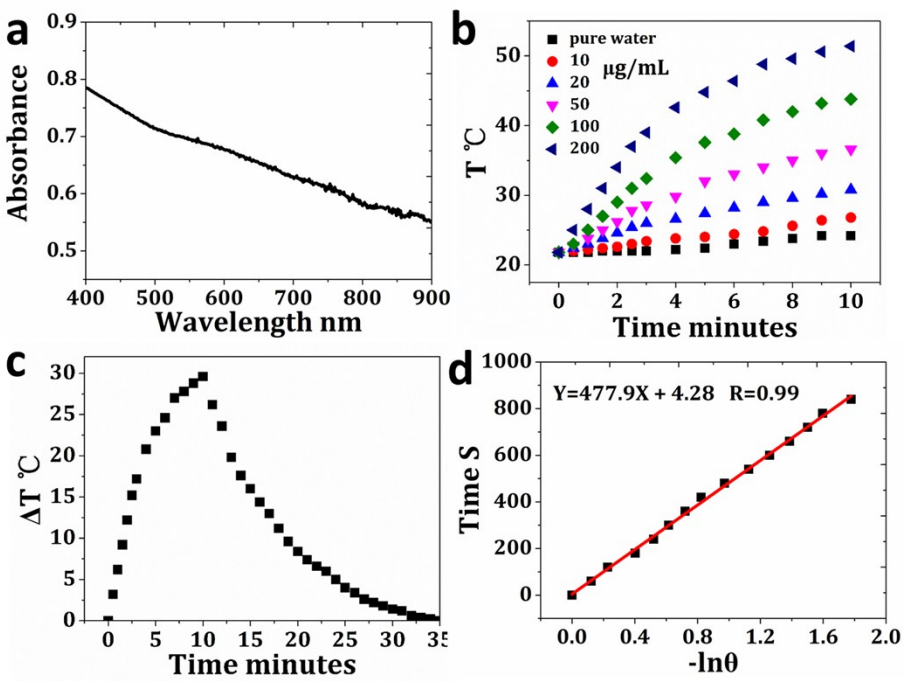

Figure S1. (a) The UV-vis spectra of Au@Pt nanodendrites; (b) emperature elevation of Au@Pt nanodendrites aqueous dispersions with different concentrations *versus* the laser irradiating time by a 808 nm laser (0.71 W/cm^{-2}); (c) Photothermal effect of Au@Pt nanodendrites aqueous dispersion irradiated by a 808 nm laser (0.71 W cm^{-2}). The laser was shut off after irradiation 10 minutes; (d) Linear time data from the cooling period versus negative natural logarithm of driving force temperature

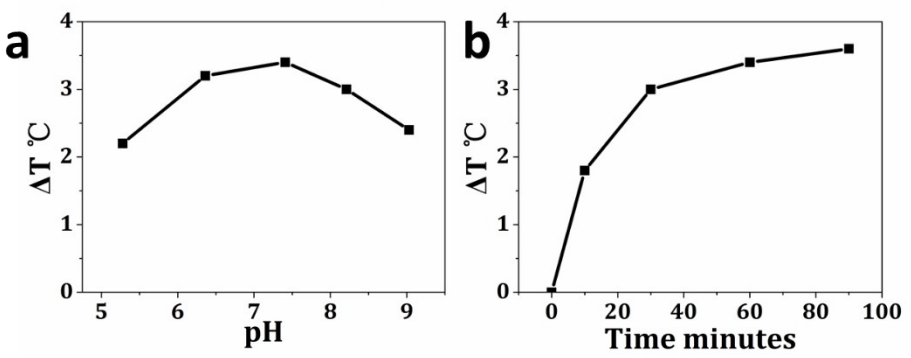

Figure S2 The optimized experimental conditions of photochermal immunosensor: (a) pH; (b) incubation time

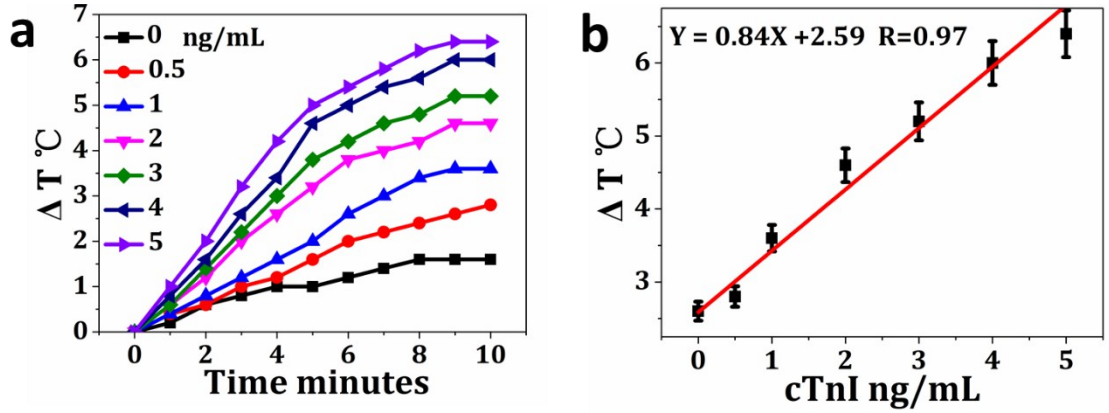

Figure S3. (a) The temperature curves of Au@Pt nanodendrites enhanced photothermal immunosensor for the detection of different cTnI concentrations; (b) the calibration curve by Au@Pt nanodendrites enhanced photothermal immunosensor.

Table S1 Comparison with other reported cTnI immunosensors

methods	Detection ranges	Detection limits	references
electrochemical immunosensor	0.05-1 ng/mL	0.05 ng/mL	4
electrochemical immunosensor	0.01-10 ng/mL	4.2 pg/mL	5
electrochemiluminescence immunosensor	2.5 pg/mL-10 ng/mL	2 pg/mL	6
electrochemical immunosensor	0.01-1 ng/mL	0.01 ng/mL	7
fluorescent immunosensor	2 pg/mL-10 ng/mL	2 pg/mL	8
photoelectrochemical immunosensor	5 pg/mL-20 ng/mL	1.756 pg/mL	9
electrochemiluminescence immunosensor	0.1-1000 ng/mL	0.06 ng/mL	10

ratiometric fluorescent immunosensor	0.1-1000 pg/mL	0.056 pg/mL	This method
colorimetric immunosensor	0.1-1000 pg/mL	0.083 pg/mL	This method
photothermal immunosensor	0.5-5 ng/mL	0.34 ng/mL	This method

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