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SUPPORTING INFORMATION

New visual immunoassay for prostate-specific antigen using near-infrared exciting Cu_xS nanocrystals and imaging on smartphone

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EXPERIMENTAL SECTION

Material and Reagent. Sodium sulfide solution ($Na_2S \cdot 9H_2O$), cupric chloride ($CuCl_2$), sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), human prostate-specific antigen (PSA) enzyme-linked immunosorbent assay (ELISA) kit (for serum, plasma, cell culture supernatant and urine; cat# no.: RAB0331) and monoclonal rabbit anti-human PSA capture antibody (clone: RM323; ELISA: 1:1000 – 1:5000; cat# no.: SAB5600147) were purchased from Sigma-Aldrich (St. Louis, MO, USA). T4 DNA ligase and Phi29 DNA polymerase were purchased from Thermo Fisher Scientific Inc. (Shanghai, China). Deoxyribonucleoside 5'-triphosphates mixture (dNTP) and adenosine 5'-triphosphate (ATP) were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). Tris(2-carboxyethl)phosphine (TCEP) was achieved from Merck KGaA (Darmstadt, Germany). All oligonucleotides used in this study were acquired from Sangon Biotech Inc. (Shanghai, China). The sequences are listed as follows:

(1) PSA aptamer-primer probe (Apt-pDNA):

5'-<u>TTATTATTAAATTAAAGCTCGCCATCAAATAGCTTTTT</u>*CCGGCCAACAC***GTTCCACACTT AC**-3'

(2) Linear padlock DNA:

5'-phosphate-*GTGTTGGCCGG*TTTTTTTTTTTTTTTTTTTTTTT**GTAAGTGTGGAAC**-3'

(3) Thiolated DNA for linkage with Cu_xS:

5'-SH-$(CH_2)_6$-TTTAAAAAAAAAAAAAAAAAAAAAA-3'

The underlined portion at the PSA-aptamer-primer probe (Apt-pDNA) is the aptamer of PSA, whereas the italicized/bold letters are the primer of rolling circle amplification (RCA) reaction. The italicized/bold portions at the linear padlock DNA (p = 5' phosphate) match with those of the Apt-pDNA, respectively.

All other chemicals were of extra pure analytical grade and used without further purification. All the solutions were prepared with ultrapure water obtained from a Milli-Q water purifying system (18.2 MΩ cm, Milli-Q, Millipore). High-binding polystyrene 96-well microplates (Ref. 655061) were purchased from Greiner (Frickenhausen, Germany). A pH 9.6 carbonate coating buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ and 0.2 g NaN_3) and a pH 7.4 phosphate-buffered saline (PBS, 10 mM) (2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.24 g KH_2PO_4, 0.2 g KCl

and 8.0 g NaCl) were prepared by adding the corresponding chemicals into 1000-mL ultrapure water, respectively. The blocking buffer and washing buffer were obtained by adding 1.0 wt % BSA and 0.05% Tween 20 (v/v) in PBS, respectively. The DNA ligation buffer and reaction buffer were prepared as follows:

DNA ligation buffer: pH 7.8, 10 mM $MgCl_2$, 40 mM Tris-HCl, 10 mM dithiothreitol (DTT), 0.5 mM ATP;

Reaction buffer: pH 7.9, 10 mM $Mg(Ac)_2$, 33 mM Tris-HAc, 66 mM KAc, 0.1% (v/v) Tween 20, 1.0 mM DTT, dNTP (500 μM), BSA (0.1 mg mL^{-1}).

Preparation of Cu_xS-DNA Conjugates. Prior to conjugation, Cu_xS nanocrystals (Cu_xS NCs) were first prepared consulting to the previous report with minor modification.[1] Initially, 250 μL of Na_2S aqueous solution (1.0 M) was added into 250 mL of the mixture containing $CuCl_2$ (1.0 mmol) and sodium citrate (0.68 mmol). After being stirred for 10 min at room temperature, the mixture was initially heated to 90 °C for 20 min and then cooled naturally to room temperature. During this process, Cu_xS nanocrystals were synthesized with the dark green color. Following that, the resulting suspension was centrifuged for 15 min at room temperature (15,000*g*), and the obtained pellets was dispersed into ultrapure water (2.0 mL).

Next, Cu_xS nanocrystals were used for the labeling of the thiolated DNA similar to previous report.[2] Firstly, 100 μL of 20-μM thiolated DNA was mixed with 5.0 μL of 2.0 mM TCEP for 30 min in order to break the disulfide bond. Subsequently, the mixture was dropped into the above-prepared Cu_xS suspension (1.0 mL) in the dark to form the Cu_xS-DNA conjugates *via* the Cu-S bond.[2] Finally, Cu_xS-DNA conjugates were centrifuged for 15 min at 15,000*g* (4 °C). The obtained pellets were dispersed in PBS (1.0 mL, 10 mM, pH 7.4), and stored at 4 °C when not in use.

Calculation of Photothermal Conversion Efficiency. The experiment was carried out in a transparent quartz pool (~5.231 g) with the above-prepared Cu_xS nanocrystals (1.0 g). The photothermal conversion efficiency (η) of Cu_xS nanocrystals was calculated according to these literatures.[3,4] Detailed calculation was given as the following equations on the basis of total energy balance in this system:

$$\sum_i m_i C_{p,i} \frac{dT}{dt} = Q_{NPS} + Q_s - Q_{loss} \quad (1)$$

where m_i and $C_{p,i}$ are the mass and heat capacity of solvent or nanoparticles, respectively. T is the measured solution temperature.

Q_{NPS} is the absorbed photothermal energy by Cu_xS NPs:

$$Q_{NPS} = I(1 - 10^{-A_\lambda})\eta \quad (2)$$

where I is the laser power, A_λ is the absorbance of Cu_xS aqueous solution under 808 nm excitation, and η is the photothermal conversion efficiency.

Q_{loss} is the lost thermal energy to the environment:

$$Q_{loss} = hA\,\Delta T \quad (3)$$

Where h is the heat transfer coefficient, A is the container's surface area, and ΔT is equal to the T (solution temperature) minus T_{surr} (ambient temperature).

Q_s is the heat absorption by solvent per second, which is the heat input of pure water, equaling to the heat output at the maximum steady-statue temperature:

$$Q_s = Q_{loss} = hA\,\Delta T_{max,H_2O} \quad (4)$$

Where $\Delta T_{max,H_2O}$ is the temperature change of water under the maximum steady-state temperature.

At the maximum steady-statue temperature, the mixture (water and Cu_xS NPs) heat input equal to the heat output, therefore, the equation can be:

$$Q_{NPS} + Q_s = Q_{loss} = hA\,\Delta T_{max,mix} \quad (5)$$

Combining the equation (2) with (4) and (5), η can also be described as follows:

$$\eta = \frac{hA\,\Delta T_{max,mix} - hA\,\Delta T_{max,H_2O}}{I\left(1 - 10^{-A_\lambda}\right)} = \frac{hA\left(\Delta T_{max,mix} - \Delta T_{max,H_2O}\right)}{I\left(1 - 10^{-A_\lambda}\right)} \quad (6)$$

where hA is the unknown. To get the hA, we present θ defined as follows:

$$\theta = \frac{\Delta T}{\Delta T_{max}} \quad (7)$$

Bring formula (7) into (1):

$$\frac{d\theta}{dt} = \frac{hA}{\sum_i m_i C_{p,i}}[\frac{Q_{NPS} + Q_s}{hA\,\Delta T_{max}} - \theta] \quad (8)$$

When the laser was off, the $Q_{NPS} + Q_s = 0$, equation (8) could be described to:

$$dt = \frac{\sum_i m_i C_{p,i}}{hA} \frac{d\theta}{\theta} \quad (9)$$

Convert (9) into the expression:

$$t = -\frac{\sum_i m_i C_{p,i}}{hA} ln\theta \quad (10)$$

Where $\frac{\sum_i m_i C_{p,i}}{hA}$ can be obtained by linear relationship between t and -ln(θ). m_{H2O} was 1.0 g, C_{H2O} was 4.2 J g^{-1} °C^{-1}. So we can get hA equals 10.9 mW °C^{-1}. We put I = 800 mW, A_{808} = 1.33, $\Delta T_{max,mix} - \Delta T_{max,H_2O} = 21.9\ °C,$ into Eq. 6, the photothermal transduction efficiency is determined to be 31.3%.

Immunoreaction and Visual Photothermal Immune-Imaging Assay. The sandwich-type immunoreaction was implemented in the microtiter plate.[5] A high-binding polystyrene 96-well microplate (Ref. 655061, Greiner, Frickenhausen, Germany) was coated overnight at 4 °C with 50 μL per well of monoclonal anti-human PSA capture antibody at a concentration of 10 μg mL^{-1} in 0.05 M sodium carbonate buffer (pH 9.6). The microplate was covered with adhesive plastic plate sealing film to prevent evaporation. On the following day, the plate was washed three time with washing buffer, and then incubated with 300 μL per well of blocking buffer for 60 min at 37 °C with shaking. The plate was then washed as before. Following that, 50 μL of PSA standards or samples with various concentrations was added into the microplate, and incubated for 60 min 37 °C under shaking. After washing with the washing buffer, 50 μL of PSA aptamer-primer probe (Apt-pDNA, 0.5 μM) was dropped into the wells and incubated for 90 min at 37 °C (note: capture antibody and Apt-pDNA sandwiched the target PSA to form a

complex with the primer for RCA reaction). After washing again, 50 μL of DNA ligation buffer including padlock DNA (0.1 μM) and 40 U T4 DNA ligase were added into the above well and incubated at 22 °C for 60 min. Subsequently, 80 μL reaction buffer containing phi29 DNA polymerase (30 U) and dNTP (500 μM) was dropped into the mixture, incubated for 45 min at 37 °C and terminated by removing the reaction solution with ultrapure water. After that, the results were mixed with 100 μL of the as-prepared Cu_xS-DNA suspension. After reaction for 60 min at 37 °C, the redundant Cu_xS-DNA conjugates were removed by washing three times with ultrapure water, followed by the addition of 200 μL of the ultrapure water. The thermal contrast temperature range for each measurement was determined by two thermostatic objects at 20 °C and 30 °C. Then, the visual photothermal immune-imaging assay was carried out by the 808-nm laser at 0.8 W. All the data were obtained with three measurements each in parallel.

PARTICAL RESULTS AND DISCUSSION

Optimization of Experimental Conditions. To maximize the photothermal response of the NIR light triggered biosensing mode, some experimental conditions such as aptamer-PSA reaction time and RCA reaction time should be investigated. 5.0 ng mL^{-1} PSA was employed as an example to screen out the optimum experimental conditions. Typically, the incubation time between PSA and aptamer influenced the combination effect. As shown from Fig. S3-A, the temperature response increased with the increasing reaction time and tended to level off after 90 min. In order to save assembly time, 90 min was chosen for PSA-aptamer reaction. RCA reaction time also played a significant role in the sensitivity of the visual photothermal detection platform, because it directly influenced the content of Cu_xS in detection system. As shown in Fig. S3-B, analogically, the temperature response increased with the RCA reaction time, then arrived at a platform after 45 min. More time to reaction did not bring about an obvious temperature response. Therefore, 45 min was selected for producing the RCA results in this work.

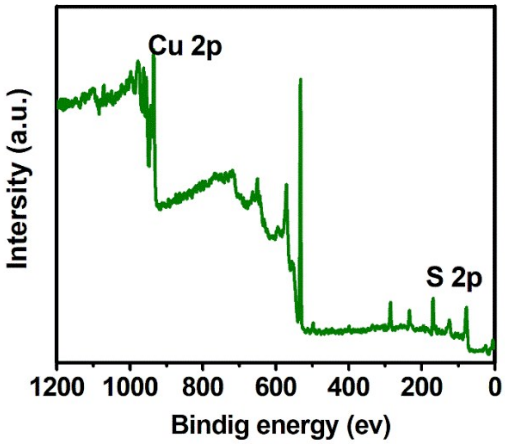

Fig. S1. XPS survey spectra of Cu_xS nanocrystals.

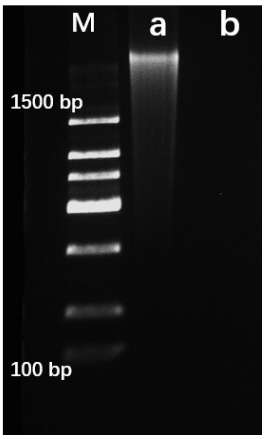

Fig. S2. Agarose gel electrophoresis of RCA product [(M): DNA marker, (a) RCA product, and (b) control experiment in the absence of aptamer-primer DNA].

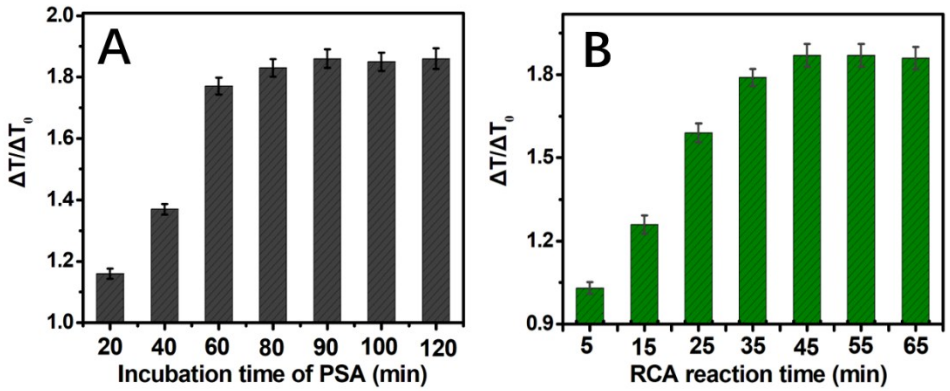

Fig. S3. Influence of (A) incubation time between target PSA and the aptamer, and (B) RCA reaction time (5.0 ng mL^{-1} PSA used as an example in this case).

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