Electronic Supplementary Information (ESI) for

A Non-Enzyme Cascade Amplification Strategy for Colorimetric Assay of Disease Biomarkers

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Experimental Section

Materials and reagents. Sodium hexachloroplatinate(IV) hexahydrate ($Na_2PtCl_6 \cdot 6H_2O$, 98%), potassium bromide (KBr, 99%), silver nitrate ($AgNO_3$, ≥99.0%), hydroquinone (≥99%), polyvinylpyrrolidone (PVP, M.W.≈55000), 3,3',5,5'-tetramethylbenzidine (TMB, >99%), hydrogen peroxide solution (wt=30% in H_2O), citrate-capped Ag nanospheres of different sizes, human prostate-specific antigen (PSA, ≥99%), acetic acid (HOAc, ≥99.7%), sodium acetate (NaOAc, ≥99%), bovine serum albumin (BSA, ≥98%), tween 20, sodium chloride (NaCl, ≥99.5%), potassium chloride (KCl, ≥99%), sodium phosphate dibasic (Na_2HPO_4, ≥99%), potassium phosphate monobasic (KH_2PO_4, ≥99%), dimethylformamide (DMF), human serum, and sulfuric acid (H_2SO_4, 95−98%) were all obtained from Sigma–Aldrich. Ethylene glycol (EG)

was from J. T. Baker. mPEG-SH (M.W.≈5,000) was obtained from Laysan Bio, Inc. Rabbit anti-PSA polyclonal antibody (rabbit anti-PSA pAb), mouse anti-PSA monoclonal antibody (mouse anti-PSA mAb), and commercial ELISA kit for PSA (product code: ab188389) were from Abcam plc. HRP-goat anti-mouse IgG conjugate and goat anti-mouse IgG were obtained from Thermo Fisher Scientific, Inc. 96-well microtiter plate (polystyrene, clear, flat bottom) was obtained from Celltreat Inc. All aqueous solutions were prepared using deionized (DI) water with a resistivity of 18.0 MΩ·cm.

Synthesis of Pt nanocubes. Pt nanocubes with an average edge length of 7.3 nm (Fig. 2b) were synthesized according to our recently published method with slight modifications.[S1] Briefly, 3.5 mL EG solution containing 20 mg of KBr and 40 mg of PVP was added to a 20-mL glass vial and heated to 180 °C in an oil bath for 10 min under magnetic stirring. Then, 0.5 mL $Na_2PtCl_6 \cdot 6H_2O$ (20 mg/mL, in EG) was added to the reaction solution. The reaction was allowed to proceed at 180 °C for 20 min and cooled to room temperature with an ice bath. The products were collected by centrifugation and stored in DI water for future use. The concentration of final Pt nanocube suspension was estimated to be 334 nM (*i.e.*, 2.01×10^{14} particles/mL).

Note that all particle concentrations in this work were determined by the combination of ICP-OES analysis and TEM imaging. See the following section "Characterizations" for details.

Demonstration of the non-enzyme cascade amplification (NECA) system (see Fig. 2). 50 μL aqueous suspensions of citrate-capped 20 nm Ag nanospheres with different particle concentrations were mixed with 50 μL of 0.1 M H_2O_2 in a cuvette. After incubation at 37 °C in an oven for 20 min, the etching solution was taken out to room temperature. Then, to the etching solution, 20 μL of PVP-capped 7.3 nm Pt nanocubes (100 pM, in DI water) and 100 μL of TMB substrate solution (*i.e.*, 4 M H_2O_2 and 1.6 mM TMB in 20 mM NaOAc/HOAc buffer, pH 4.0) were sequentially added. After incubation at room temperature for 20 min, the catalytic reaction was stopped by adding 20 μL of 2 M H_2SO_4, in which oxidized TMB was converted to yellow-colored diimine with $\lambda_{max} \approx 450$ nm and $\varepsilon = 5.9 \times 10^4$ $M^{-1} \cdot cm^{-1}$.[S2] UV-vis spectra were then taken from the reaction solutions using a spectrophotometer.

Preparation of Ag nanosphere-goat anti-mouse IgG conjugates.[S3] A 5 μL aliquot of goat anti-mouse IgG (1 mg/mL, correspond to the "secondary antibody" in Fig. 1) was mixed with 10 μL of mPEG-SH (10 μM) at room temperature. Then, 1 mL of Ag nanospheres (0.185 mM in Ag

element) was added to the mixture and incubated at room temperature for 20 min, followed by the addition of 100 μL of BSA (1 wt%) and incubation at room temperature for another 20 min. The final conjugates were purified by washing twice with 0.1 wt% BSA and stored in 10 mM phosphate buffered saline pH 7.4 (PBS) containing 10 wt% BSA and 0.05 wt% tween 20 at 4 °C for future use.

NECA assay of PSA. In a standard NECA assay of PSA, 100 μL aliquots of rabbit anti-PSA pAb (5 μg/mL, in PBS, correspond to the "capture antibody" in Fig. 1) was added to the wells of 96-well microtiter plate and incubated at 4 °C for 12 h. After being washed by washing buffer (PBS containing 0.05 wt% tween 20) for three times, the wells were blocked by 200 μL of blocking buffer (PBS containing 0.05 wt% tween 20 and 2 wt% BSA) and incubated at 37 °C for 1 h. Then, the wells were washed three times by washing buffer, followed by addition of 100 μL of PSA standards in dilution buffer (PBS containing 0.05 wt% tween 20 and 0.5 wt% BSA). After being shaken on a shaker at room temperature for 2 h, the wells were washed by washing buffer for three times. 100 μL of mouse anti-PSA mAb (2 μg/mL, in dilution buffer, correspond to the "detection antibody" in Fig. 1) was added to the wells, followed by shaking at room temperature for 1 h. After the wells had been washed three times by washing buffer, 100 μL of Ag nanosphere-goat anti-mouse IgG conjugates (20 μg/mL in Ag elements) was added, followed by 1 h shaking at room temperature. After three times washing by washing buffer, 100 μL of H_2O_2 (0.05 M) was added to the wells, followed by incubation at 37 °C for 20 min. Finally, 20 μL of Pt nanocubes (100 pM, in DI water) and 100 μL of TMB substrate solution (4 M H_2O_2 and 1.6 mM TMB in 20 mM NaOAc/HOAc buffer, pH 4.0) were sequentially added. After incubation at room temperature for 20 min, the reaction was stopped by adding 20 μL of 2 M H_2SO_4. The absorbance of each well at 450 nm was read using a microplate reader.

PSA spiked human serum samples were measured by the same procedure for PSA standards as described above, except that the serum samples were pre-diluted 2 folds by dilution buffer prior to detection. Note that the original serum sample (from Sigma Aldrich) does not contain detectable PSA as determined by a commercial ELISA kit (Abcam plc., United Kingdom).

HRP ELISA of PSA. The detection principle and procedure of HRP ELISA was similar to those of NECA assay except that the color signal was generated from HRP. In brief, after the capture antibodies, PSA, and detection antibodies had been adsorbed on the 96-well microtiter plate (using the same procedure for NECA assay), 100 μL of HRP-goat anti-mouse IgG

conjugates (1 μg/mL, in dilution buffer) was added to each well. The plate was shaken at room temperature for 1 h and then washed three times by washing buffer. 100 μL of TMB substrate for HRP ELISA (1 mM H_2O_2 and 0.8 mM TMB in 20 mM NaOAc/HOAc buffer solution, pH 4.0) was added to each well, followed by incubation at room temperature for 20 min. The reaction at the wells were finally stopped by 20 μL of 2 M H_2SO_4. The absorbance of each well at 450 nm was read using a microplate reader.

Silver enhancement technique-coupled NECA assay of PSA. The procedure of silver enhancement technique-coupled NECA assay of PSA was same as that of above mentioned standard NECA assay except for the involvement of a silver enhancement process before the step of Ag particle etching by H_2O_2. The silver enhancement was conducted by adding 50 μL of $AgNO_3$ (0.1 mM, in DI water) and 50 μL of hydroquinone (0.1 mM, in DI water) to each well. After incubation at room temperature for 20 min, the wells were washed five times by DI water.

Characterizations. The UV-vis spectra were recorded using an Agilent Cary 60 UV-vis spectrophotometer. Transmission electron microscopy (TEM) images were taken using a JEOL microscope (JEM-2010) operated at 200 kV. Scanning electron microscopy (SEM) images were taken using a cold field emission high resolution scanning electron microscope (Hitachi S-4700) operated at 20 kV. To estimate particle concentrations, the Ag nanospheres and Pt nanocubes were dissolved to Ag and Pt ions by aqua regia. The concentrations of Ag and Pt ions were then determined using an inductively coupled plasma-optical emission spectroscopy (ICP-OES, PerkinElmer Optima 7000DV), which could be converted to the particle concentrations of Ag nanospheres and Pt nanocubes once the particle sizes and shapes had been resolved by TEM or SEM imaging.[S4,S5] A microplate reader (PerkinElmer Victor 3 1420) was used to measure the absorbance at 450 nm for samples in microtiter plates. 37 °C-incubation was conducted in an oven (Precision Scientific, Inc.). Microtiter plates were shaken on a Corning LSE digital microplate shaker. A benchtop meter (Oakton pH 700) was used to measure the pH value of buffer solutions.

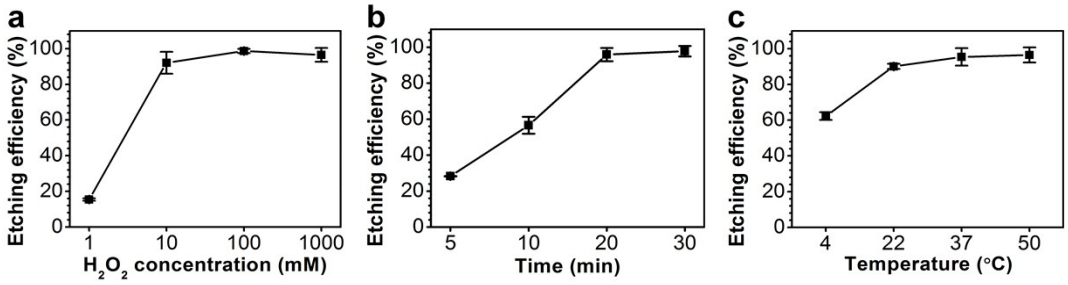

Fig. S1. The effects of (a) H_2O_2 concentration, (b) etching time, and (c) temperature on the etching efficiency of Ag nanospheres by H_2O_2. The experiments were carried out by etching 50 μL aqueous suspensions of 20 nm Ag nanospheres (~10 fM) with 50 μL of H_2O_2. The H_2O_2 concentration was 0.1 M, etching time was 20 minutes, temperature was 37 °C, unless otherwise stated. The etching efficiency is defined as the amount of released Ag^+ ions (measured by a previously reported method[S6]) as a percentage of the amount of total Ag element in initial Ag nanospheres.

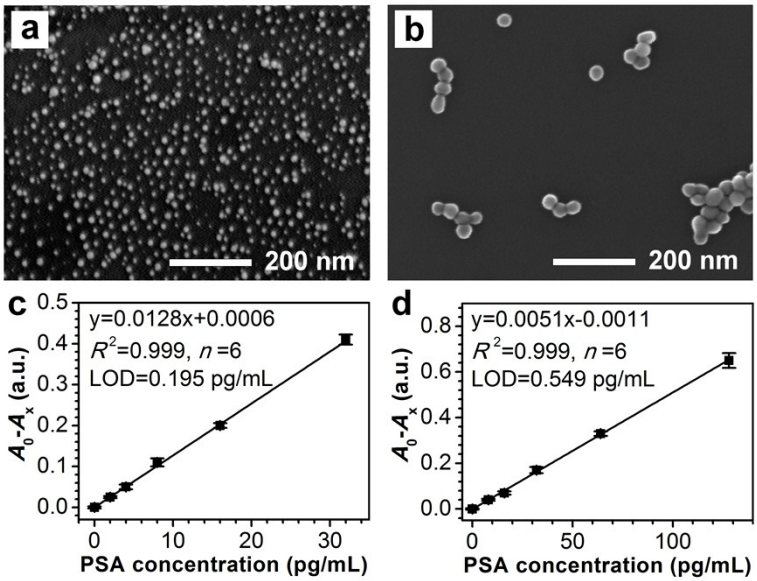

Fig. S2. Effect of Ag nanosphere size on the detection sensitivity of NECA for PSA. SEM images of Ag nanospheres with average diameters of (a) 10 nm and (b) 40 nm. Calibration curves of NECA assays when (c) 10 nm and (d) 40 nm Ag nanospheres shown in (a, b) were used. Error bars in (c, d) indicate the standard deviations of six independent measurements.

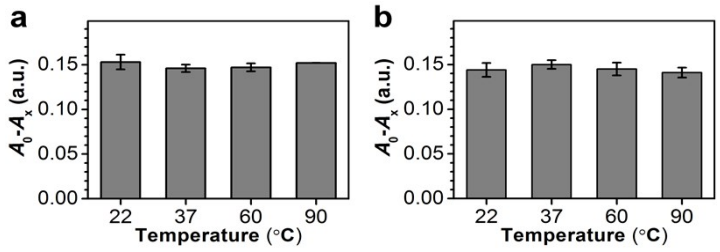

Fig. S3. Stability test for the NECA system, in which the 20 nm Ag nanospheres (a) and 7.3 nm Pt nanocubes (b) were heated at different temperatures for 6 hours before they had been utilized for the NECA assay of 10 pg/mL PSA standards.

Table S1. Analytical recoveries of the NECA assay in detecting PSA spiked human serum samples.

PSA amount spiked (pg/mL)	PSA amount measured (pg/mL)	Coefficient of variations (%, n = 6)	Recovery (%)
5	4.68	10.91	93.62
10	9.89	8.52	98.87
20	20.86	4.22	104.31
35	32.36	5.37	92.46
50	49.76	2.78	99.52

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