Electronic supplementary information

Strain-induced PtCu nanozymes for paper-based portable colorimetric immunoassay of carcinoembryonic antigen with smartphone readout

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TABLE OF CONTENTS

Chemical and reagent. S3

Instruments and equipment S3

Assessment of nanozyme activity S4

Data stream acquisition and processing S4

ELISA testing S5

Preparation of human serum specimens S5

Figure S1 ..... S6

Figure S2 ..... S6

Figure S3 ..... S7

Figure S4 ..... S7

Figure S5 ..... S8

Figure S6 ..... S8

Figure S7 ..... S9

Figure S8 ..... S9

Figure S9 ..... S10

Figure S10 ..... S10

Chemical and reagent. Monoclonal rabbit anti-human carcinoembryonic antigen (CEA) capture antibody (denoted as mAb_1; clone no.: EPCEAR7; unconjugated; cat# ab133633), polyclonal rabbit anti-human CEA detection antibody (denoted as pAb_2; unconjugated; cat# ab131070), and human CEA enzyme-linked immunosorbent assay (ELISA) kits including CEA standards with different concentrations (cat# ab264604; one-wash 90 min protocol; sensitivity: 24.68 pg/mL; range: 78.13 - 5000 pg/mL; detection method: colorimetric; assay type: sandwich, quantitative; reaction with: human) were purchased from Abcam (Shanghai, China). All chemicals and consumables used in this work were purchased from standard commercial suppliers, including platinum(II) acetylacetonate ($Pt(acac)_2$), copper(II) acetylacetonate ($Cu(acac)_2$), dodecyltrimethylammonium bromide (DTAB), ascorbic acid, (3-aminopropyl)triethoxysilane (APTES), glutaraldehyde, bovine serum albumin (BSA), 3,3′,5,5′-tetramethylbenzidine (TMB), hydrogen peroxide (H_2O_2), and Tween 20, which were all obtained from Sigma-Aldrich; common solvents and buffer reagents including N,N-dimethylformamide (DMF), acetone, ethanol, isopropyl alcohol (IPA), and phosphate-buffered saline (PBS) were purchased from Aladdin Industrial Corporation; Sharpie Metallic Permanent Markers were purchased from Newell Brands; high-temperature V2 resin for 3D printing was supplied by Formlabs; and Rust-Oleum Painters Touch 2× Matte White Spray Paint was obtained from Rust-Oleum Corporation, while all reagents were of analytical grade and used as received without further purification.

Instruments and equipment. The testing and experimental instruments employed in this work were sourced from standard professional manufacturers: transmission electron microscopy (TEM) and high-resolution TEM (HR-TEM) were supplied by JEOL Ltd. (Japan); the ultraviolet-visible (UV-vis) spectrophotometer was purchased from Shimadzu Corporation (Japan). The in-situ attenuated total reflection Fourier transform infrared (ATR-FTIR) spectrometer was obtained from Thermo Fisher Scientific Inc. (USA). The Form 3B 3D printer for device fabrication was manufactured by Formlabs Inc. (USA). The stainless-steel high-pressure autoclave for nanozyme synthesis was provided by Parr Instrument Company (USA). The high-speed centrifuge for sample purification was acquired from Eppendorf AG (Germany). The ultrasonic cleaner for material dispersion was supplied by Branson Ultrasonics Corporation (USA). The constant-temperature incubator for immunoreactions was manufactured by Memmert GmbH + Co. KG (Germany).

Assessment of nanozyme activity. The POD-like enzyme activity was determined by comparing the oxidative discoloration of TMB over time.[1] Specifically, 100 μL (5.0 μg mL^{-1}) of the nanozyme substrate was mixed with preconfigured different concentrations of H_2O_2 (100 μL, 3.33 M) and TMB (100 μL, 10 mM). The absorbance variation of the test system was recorded under the specific modulation parameters of UV-vis. The temperature of the entire reaction system was set to 37 °C. The specific activity (SA) of POD nanozyme was calculated by the following equation:

$$SA = \frac{(V/KL) \times (A/T)}{m}$$

where V is the total volume of the test system (μL); k is the molar absorption coefficient of the colorimetric substrate, which for TMB typically reaches a maximum at 39K $M^{-1}cm^{-1}$ at 652 nm; L is replaced by the path length of the light propagating in the cuvette (cm); A is the absorbance in the test system after deducting the absorbance; the ratio of A to T is the initial rate of change of the absorbance at 652 nm (min^{-1}); and m is the weight of the nanoparticle enzyme for each assay (mg). In addition, the molecular part of the fractional equation represents the catalytic reaction rate per unit of nanozyme. According to this, combined with the reaction rate curves for different concentrations of substrates, the Mie equation could be obtained. Further, the double inverse curve was used to further determine km and vmax values according to the following equation:

$$v = (v_{max} \times [S])/(K_m + [S])$$

Where, S represents the substrate concentration, vmax represents the maximum reaction rate, and Km represents Michaelis constant. km reacts the affinity between the nanozyme and the substrate.

Data stream acquisition and processing. All colorimetric images were captured using a smartphone (iPhone 14pro, Apple Inc.) under a fixed illumination environment. The shooting distance between the smartphone camera and the paper-based device was maintained at approximately 15 cm, and all images were acquired at a vertical angle to minimize optical deviations. A white background and constant LED illumination box were employed throughout the experiments to ensure uniform lighting conditions. The acquired images were analyzed using color-analysis software. The RGB signals from each reaction zone were extracted under identical processing parameters, and the grayscale intensity was calculated using the averaged pixel values from five independent regions. The entire image acquisition and processing workflow was standardized to improve analytical reproducibility

ELISA testing. Commercial standard method testing for CEA was performed by using custom kit. A monoclonal capture antibody specific for Human CEA was pre-coated onto a 96-plate, and when a standard or sample were added, the Human CEA therein would bind to the capture antibody. When horseradish peroxidase-labeled human CEA antibody was added, horseradish peroxidase-labeled human CEA antibody binds to human CEA to form a sandwich immune complex. Finally, by adding the color developer TMB solution, the solid-phase captured horseradish peroxidase then catalyzed the oxidation of the colorless color developer to a blue substance, which became yellow after the addition of the termination solution. Quantitative detection was achieved by detecting the absorbance value at 450 nm by a microplate reader. Generally, blank, gradient concentration samples and samples to be tested were added to the pre-prepared blank wells, standard wells and real sample test wells respectively and incubated in a 37 ℃ thermostat for 30 min. Afterwards, the incompletely captured CEA molecules were washed by PBS washing solution and repeated several times. Afterwards, 50 μL of enzyme labeling reagent was added to it. Following this, chromogenic agents A, and B respectively were added to the microplates and continued to be incubated at 37 °C for 10 minutes. Finally, 50 μL of termination solution is added to the wells to terminate the reaction, and the process changes from blue (ox-TMB) to yellow for high concentrations of CEA samples. Finally, the absorbance at 450 nm was recorded on an enzyme calibrator.

Preparation of human serum specimens. Prior to measurement, these collected human serum samples from Fujian Medical University Union Hospital (Fuzhou, China) were initially centrifuged for 5 min at 4 ^{o}C with 5,000*g* (centrifugal force) to remove the possibly existed impurities and macromolecules. Thereafter, the obtained supernatant fluids were determined using the developed immunoassay and the commercialized human CEA ELISA kit, respectively.

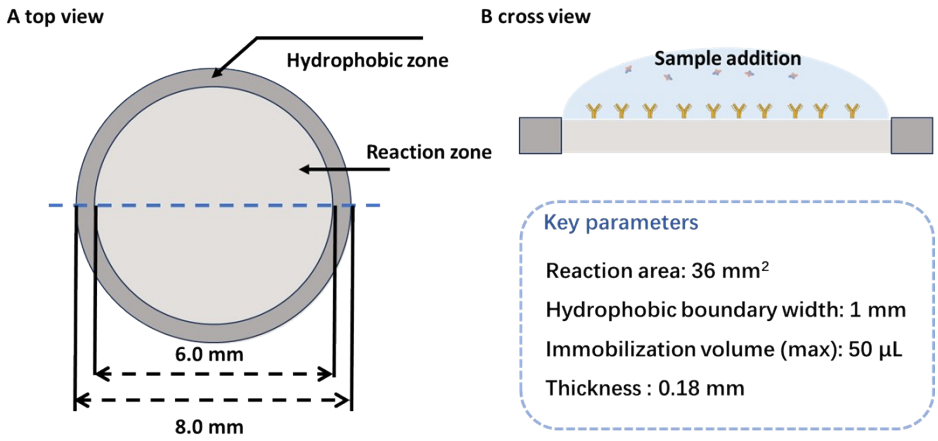

Fig. S1 Schematic diagram and key parameters of the core component of a paper-based colorimetric immunoassay sensor.

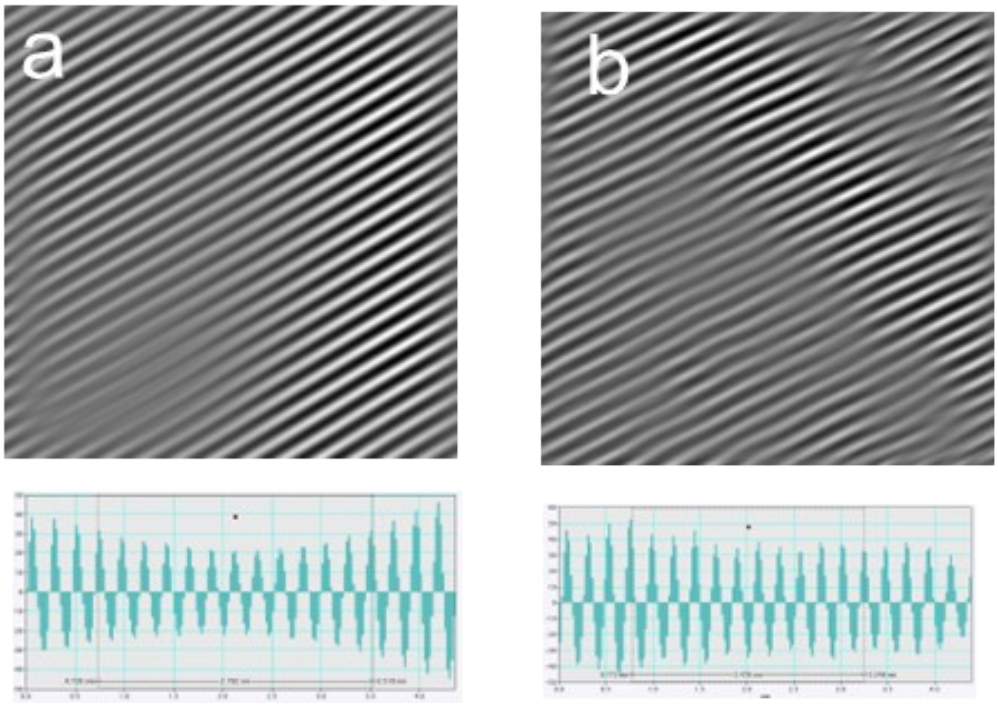

Fig. S2 Fourier transform images of the corresponding high-resolution regions of PtCu and images showing the measured dimensions.

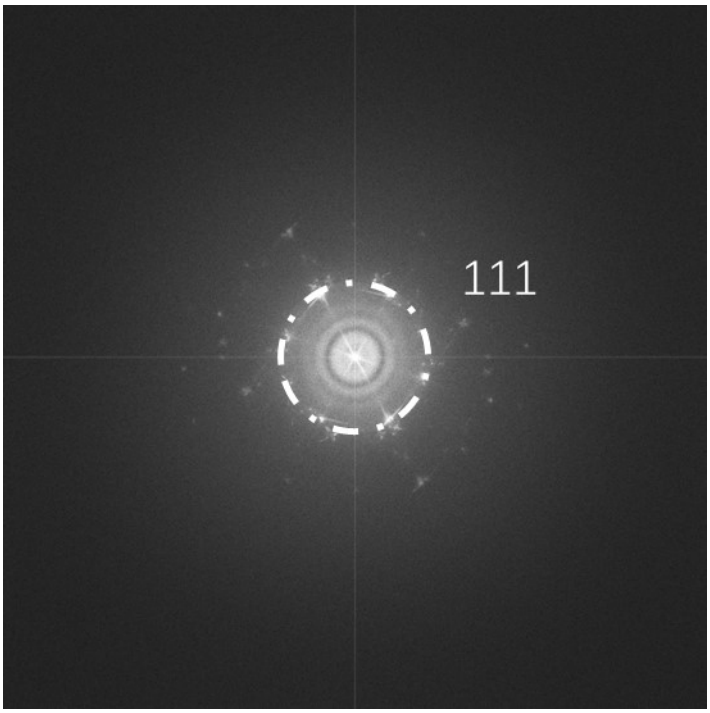

Fig. S3 High-resolution TEM FFT image of PtCu.

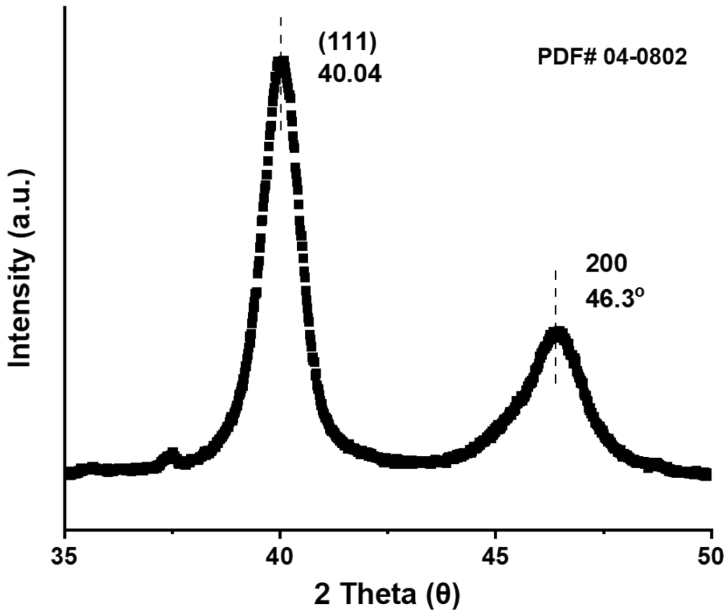

Fig. S4 X-ray diffraction patterns of the synthesized PtCu nanozymes, where the dashed lines indicate the diffraction angles of the 111 and 200 planes from the standard pattern of the Pt nanozyme. A large shift in the diffraction angle indicates the introduction of submicron-sized Cu and the resulting compressive strain.

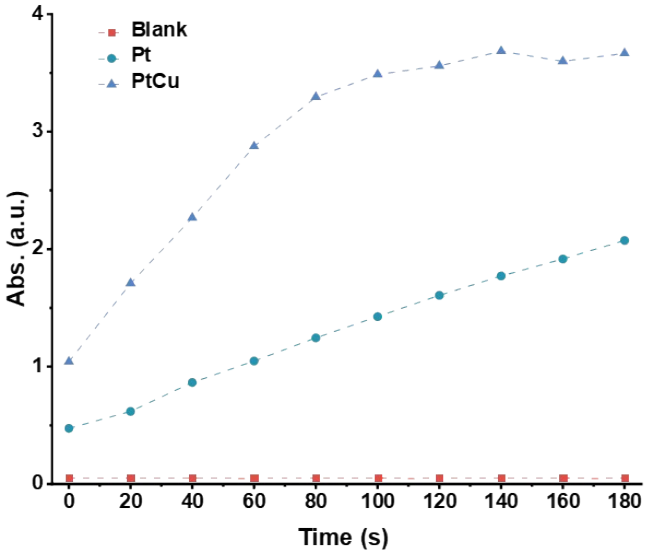

Fig. S5 Time-dependent absorbance curves of Pt and PtCu catalysts.

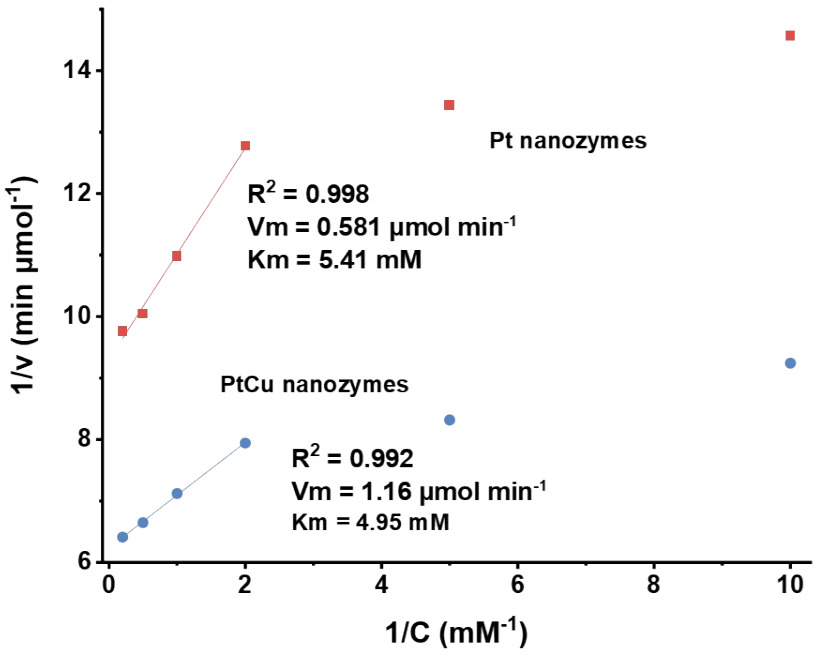

Fig. S6 Double-reciprocal fit of the catalytic reaction rates for Pt and PtCu nanocatalysts.

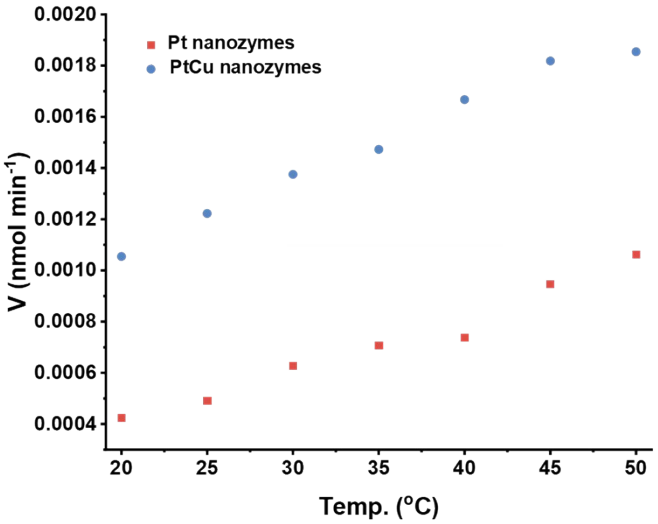

Fig. S7 Reaction rate curves for Pt and PtCu catalysts at different temperatures.

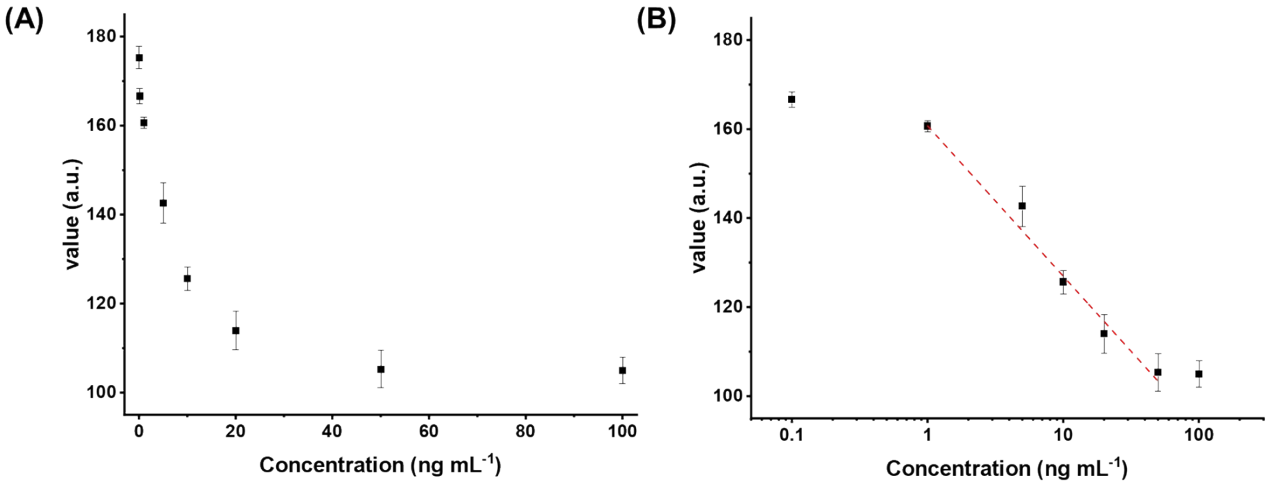

Fig. S8 (A) Gray-scale values of micrographs of paper treated with different concentrations of CEA; (B) Fitting curves corresponding to the grayscale values of micrographs of paper exposed to different concentrations of CEA.

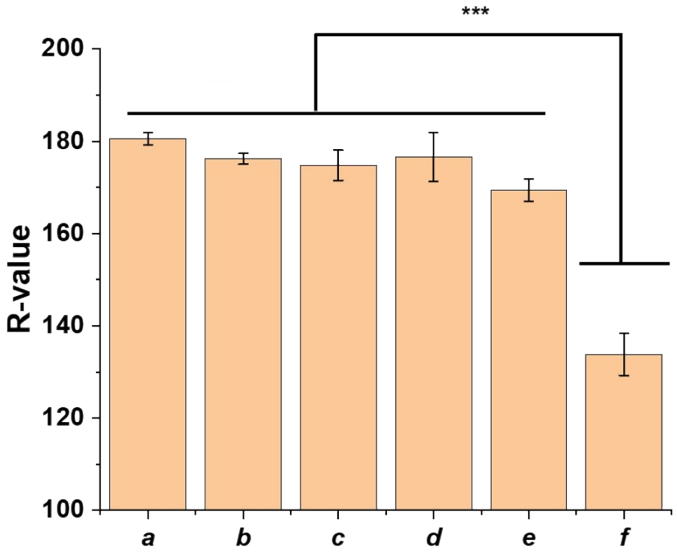

Fig. S9 Statistical bar chart of color values on paper for different interferents and targets.

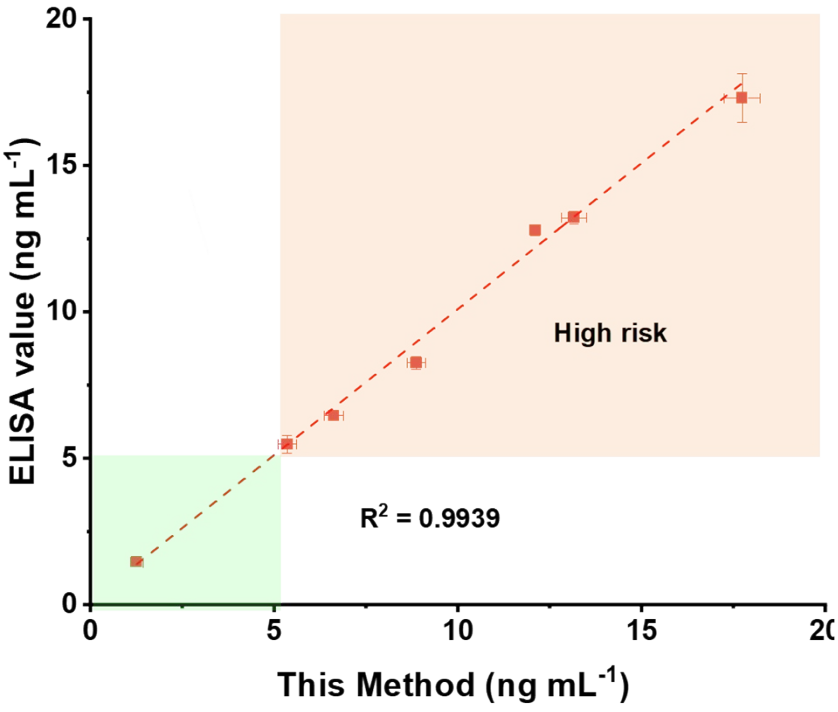

Fig. S10 Results of a double-blind trial based on a commercially available ELISA assay and a newly developed colorimetric assay.