Catalytic activity-modulated etching of plasmonic gold nanorods enabled ultra-sensitive colorimetric strategy for Early-Stage cancer diagnosis

Caiping Ding a, Ming Li a, Weiwei Chen a, Zikang Chen a, Zhanhu Guo b, Ben Bin Xu b,*, Xuehua Zhang c, Zhiyong Guo d, Youju Huang a,*

a College of Material Chemistry and Chemical Engineering, Key Laboratory of Organosilicon Chemistry and Material Technology, Ministry of Education, Key Laboratory of Organosilicon Material Technology of Zhejiang Province, Hangzhou Normal University, Hangzhou 311121, Zhejiang, China
b Mechanical and Construction Engineering, Faculty of Engineering and Environment, Northumbria University, Newcastle upon Tyne NE1 8ST, U.K
q Department of Chemical and Materials Engineering, University of Alberta, T6G 1H9, Edmonton, Alberta, Canada
d State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, State Key Laboratory Base of Novel Functional Materials and Preparation Science, School of Materials Science and Chemical Engineering, Ningbo University, Ningbo 315211, Zhejiang, China

* Corresponding authors.
E-mail addresses: Ben.xu@northumbria.ac.uk (B.B. Xu), yjhuang@hznu.edu.cn (Y. Huang).

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ABSTRACT

While the aberrant low expression of metallothioneins (MTs) in tissue has been recognized as an effective indicator to spot the occurrence of early-stage malignant tumors, the ultra-sensitive detection of MTs at a low concentration (<10 nM) remains to be developed. Here, a facile multicolor colorimetric visualization strategy is introduced to detect MTs at an extremely low concentration down to 28 pM, enabled by the catalytic activity-modulated etching of plasmonic gold nanorods (CAEPAu). The catalytic reduction is inhibited by covering the nanospheres with highly specific protein corona recognizing MTs, thus facilitating a customized etching of nanorods (NRs) into nanospheres to present color correspondingly. The CAEPAu assay detects MTs in 35 min, significantly faster than the existing method (~3 h). We demonstrate to use CAEPAu assay to precisely detect and distinguish MTs in four tumor cells, clinic blood samples and other proteins.

1. Introduction

The metallothioneins (MTs) are a group of intracellular proteins with a very low molecular weight (6–7 kDa) and a large cysteine concentration. The MTs play an essential role in scavenging free radicals, regulating intracellular metal ion homeostasis, maintaining homeostasis in redox reaction, and promoting cell proliferation and apoptosis [1–3]. MTs have been recognized as effective indicators for heavy metal detoxification and environmental pollution [4–8]. More importantly, the expression of MTs has been closely associated with tumor occurrence and development at an early-stage [9]. Such abnormally expressed MTs usually exist at low concentrations during the initial stages of carcinogenesis [10,11]. The detection of these MTs remains challenging by conventional protein testing techniques [12,13], such as enzyme linked immunosorbent assay (ELISA), due to the inaccurate expression and insensitivity at a low MTs level. From the perspective of knowledge, the relationship between tumor progression and high MTs concentrations has been scaled with linear trends. However, the understanding of early-stage cancer development under a low concentration of MTs has been less explored [14,15]. The latest exercise suggested that the critical level of MTs in early-stage malignant breast cancer is very low (~0.56 nM) [16], so it is highly desired to unveil the secret of abnormal expression of MTs at a low concentration scale.

A number of MTs detection technologies have been invented, including immunological assays (50 nM) [17], electrochemistry (16.7 nM) [18], mass spectrometry (50 nM) [19], colorimetry (10.49 nM) [20], resonance light scattering (76.8 nM) [6], quartz crystal microbalance (3.3 nM) [21], and fluorescence (5.25 nM) [4]. Most of the above methods can only work on a MT concentration at a relatively high level (~10 nM). Quartz crystal microbalance and fluorescence methods are heavily dependent on instruments and equipment, both of which are time-consuming. Colorimetric methods have been widely used in therapeutic diagnosis and health management [22–25]. But, for various target analytes, it is imperative that simple signal readout strategies are created, transferred, amplified, and sensitized to the appropriate extent [26–28]. Researchers have developed gold nanorods (Au NRs)-based colorimetric methods for quantitative and semi-quantitative detection of MTs [29–31], however, those methods only detect MTs at relatively...
high levels (>10 nM). Therefore, developing a low-cost, rapid and convenient method for detecting abnormal MT expression within the low concentration range remains unachievable.

In this work, we propose a facile colorimetric visualization strategy for the detection of MTs at an ultra-low concentration (<10 nM). The catalytic activity-modulated etching of plasmonic gold nanorods (CAE-PAu) is carried out by controllably patching MTs onto the surface of gold nanospheres (Au NPs) catalyst unit, allowing a precisely controlled chemical reaction to take place. The etching of Au NRs can be tailored to produce an accurate detection in a visible manner (Fig. 1a). In this elaborate design, the exposed Au NPs catalyze the reduction of p-nitrophenol (p-NP) to p-aminophenol (p-AP) by NaBH₄ [32,33]. During etching, the resulting p-AP reacts with KIO₃ to further generate the etching agent I₂, which is used to produce highly vibrant, richly colored Au NRs. Target MTs adsorb effectively on exposed Au NPs to form protein corona and occupy their catalytic sites in the detection system [34–36]. In Fig. 1b, the working principle is diagrammatically shown on employing ultra-sensitive CAEPAu assay to detect cancers such as breast cancer, lung cancer, cervical cancer, and gastric cancer.

2. Materials and methods

2.1. Materials

Chloroauric acid (HAuCl₄·3H₂O, 99.9 %) and Hexadecyl trimethyl ammonium bromide (CTAB, 99.0 %) were bought from Sigma-Aldrich Co., Ltd. (Shanghai, China). Trisodium citrate dehydrate (SC, ≥99.0 %), tris(hydroxymethyl)-amino-methane (TB, GR), n-hexane (GR), sodium borohydride (NaBH₄, 99.0 %), ascorbic acid (AA, 99.7 %), silver nitrate (AgNO₃, ≥99.0 %), hydrochloric acid (HCl, 37 wt% in water), sodium bromide (NaBr, 99.0 %), potassium iodate (KIO₃, ≥99.8 %), riboflavin (RF, ≥97.3 %), terephthalic acid (TA, ≥99.0 %), p-nitrophenol (p-NP, ≥99.0 %) and p-aminophenol (p-AP, 98.0 %) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Nitrotetrazolium blue chloride (NBT) was bought from Macklin Biochemical Co., Ltd. (Shanghai, China). Metallothioneins (MTs, S12070, 95.0 %), cytochrome c (Cyt-c, S12058, 95.0 %), recombinant human serum albumin (HAS, S29747, 95.0 %), immune immunoglobulin G (IgG, S25766, 90.0 %), and bovine serum albumin (BSA, S12012, 98.0 %) were purchased from Yuanye Biotechnology Co. Ltd. (Shanghai, China). Ribonuclease A (RNAse A), transglutaminase (GGT), and human epididymal protein 4 (HE4) were purchased from Huzhen Industrial Co., Ltd (Shanghai, China). Three types of breast cancer cells (MCF-10A, MCF-7, MDA-MB 231), gastric cancer cells (Sgc-7901), lung cancer cells (H358), cervical cancer cells (Hela) samples are from laboratory, and whole blood samples from four cancer patients are from volunteers. The human metallothionein ELISA kit was purchased from Shanghai Xin Yu Biotech Co., Ltd (Shanghai, China). All chemicals were used without further purification. Deionized water was used throughout the experiment.

2.2. Characterization and equipment

UV–vis absorption spectra were performed using a TU-1810 UV–vis spectrophotometer (Purkinje General Instrument Co., Ltd, China). Transmission electron microscopy (TEM) was performed on a JEOL HT-7700 electron microscope operating at 200 kV (Hitachi, Japan). The precision acidity meter was provided by Dapu Instruments Co., Ltd. (Shanghai, China). The adjustable pipette was provided by Thermo electric Instrument Co., Ltd. (Shanghai, China). The Milliq ultrapure water was used throughout the experiment.

Fig. 1. (a) The schematic illustration of CAEPAu with unique colorimetric properties to visually detect MTs. (b) The application of MTs detection at an ultra-low concentration for cancer diagnosis. TEM images of (c) Au NPs and (d) Au NRs. (e) UV–vis absorption spectra and color photographs (insets) of bare Au NPs and Au NRs. (f) The illustration of p-NP catalysis by Au NPs, and the inhibiting effect of Au NPs catalysis by MTs corona. (g) UV–vis absorption tracking on each step of Au NPs catalytic reaction shielded by MTs with different reaction solutions (insets): (I) p-NP, (II) p-NP + NaBH₄, (III) p-NP + NaBH₄ + Au NPs, and (IV) p-NP + NaBH₄ + Au NPs + MTs, (b) The UV–vis absorption spectra of Au NRs etched under different concentrations of MTs (black line – 0 nM, pink line – 3 nM, and blue line – 5 nM) and the photos (insets). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
water system is provided by Millipore (Bedford, USA). The analytical balance was provided by Sartorius Scientific Instrument Co., Ltd. (Beijing, China).

2.3. Synthesis of Au NPs and Au NRs

According to the previously reported method [27,28], Au NPs with an average diameter of 35 nm were synthesized. Briefly, trisodium citrate (SC, 10 mL, 33 mM) was added to 140 mL of deionized water under a stirring speed of 1000 rpm (the oil bath temperature was maintained at 137 °C) for 40 min. Then, fresh HAuCl₄ (1 mL, 25 mM) was quickly injected, with no rapidly change of color observed. After 60 s, tris (hydroxymethyl)-amino-methane (TB, 5 mL, 0.1 M) was added, and the solution color started to change rapidly from colorless to light pink. The mixture was keeping at boiling state and stirring at 1000 rpm for 15 min. Then the temperature was lowered to 100 °C, and HAuCl₄ (1 mL, 25 mM) was quickly injected. The solution color changed into wine red in about 1 min. The temperature was kept at this state for 20 min. Finally, HAuCl₄ (1 mL, 25 mM) was quickly injected and kept at temperature for 20 min, and then cooled to room temperature.

By following to the literatures [16,29], Au NRs were synthesized by the gold seed-mediated growth method with slight modifications. (I) Firstly, gold seeds were prepared as follows: 0.25 mL of HAuCl₄ (0.01 M) solution and 0.60 mL of 0.01 M freshly prepared ice-cold NaBH₄ solution were added to 9.75 mL of 0.10 M CTAB solution in turn. The mixture was stirred vigorously at 1200 rpm for 2 min. Before using the reaction solution, keep the mixture at room temperature for at least 2 h. (II) The typical procedure of Au NRs preparation: firstly, 4 mL of HauCl₄ (0.01 M), 0.8 mL AgNO₃ (0.01 M), 0.64 mL ascorbic acid (0.1 M) and 1.6 mL HCl (1 M) were added to 80 mL of 0.10 M CTAB in sequence in a water bath at 28 °C and stirring at 700 rpm. Subsequently, 105 μL of the prepared gold seed solution was added. After 30 min, the solution color gradually changed to burgundy. Then it was left at 28 °C in water bath overnight. The final prepared Au NRs were purified by centrifugation three times at 10000 rpm and concentrated to about 1.4 nM for further study.

2.4. Preparation of colorimetric assay and analysis process of MTs

(I) Catalytic process: 315 μL of deionized water, 30 μL of Au NPs and 50 μL of MTs of different concentrations were added to a 2 mL centrifuge tube, followed by incubation at 25 °C for 10 min. Then, 35 μL of p-NP (1 mM), 20 μL of NaBH₄ (0.1 M) prepared in ice water and 50 μL of B-R buffer solution (0.04 M, pH 5) were sequentially added to the above solution and the mixture was saturated for 5 min. (II) Etching process: 102.5 μL B-R buffer solution (0.04 M, pH 2) containing 25 mM CTAB and 50 mM NaBr, 125 μL of the above mixed solution after catalytic reaction, 2.5 μL KIO₃ (50 mM) and 50 μL of Au NRs (1.4 nM) were added to a 0.5 mL centrifuge tube. The mixture was incubated under 50 °C water bath for 20 min for further testing.

2.5. Colorimetric assay for MTs in cancer cell samples

According to conventional treatment techniques for cell samples, the 500 μL cell sample was first freeze-dissolved at −20 °C and 37 °C for three times, each lasting 30 min. Then, the supernatant was collected after being centrifuged at 3000 rpm for 5 min. It was diluted with a pH 7.4 PBS buffer solution for subsequent testing.

2.6. Colorimetric assay for MTs in cancer clinical whole blood samples

Firstly, the 500 μL of blood was centrifuged at 5000 rpm for 10 min to remove red blood cells and other substances. Then, the supernatant was collected and the centrifugation process was repeated three times. Finally, the obtained sample was diluted 5 times in pH 7.4 PBS buffer solution and stored at 4 °C for subsequent testing.

2.7. ELISA kit for identify MTs

Essentially, this kit involves modifying a capture antibody on the substrate of a multiwell plate to recognize specific antigen MTs, followed by identifying the detection antibody for a sandwich structure. Then the capture antibody is modified by streptavidin-biotin complex (SABC complex, containing HRP) to catalyze TMB to generate TMB+ with a single change of color at 450 nm for MTs quantification.

2.8. The study on antioxidative activity of MTs

(I) Prepare starch indicator (10 g/L): 1 g starch and 5 mL water were mixed in a three-neck flask. Subsequently, 95 mL of hot water was added to stir and heat at 120 °C. When heated to boiling for 3 min, the mixed solution was cooled to room temperature for use. (II) In the centrifuge tube, 930 μL starch indicator (1 g/L), 40 μL MTs at different concentrations, 10 μL KI (20 mM), 10 μL KIO₃ (2 mM) and 10 μL HCl (3 M) were added in turns, and then mixing well. Finally, the mixed solution reacted at room temperature for 10 min.

2.9. Traditional colorimetric detection procedures for MTs

In a 0.5 mL centrifuge tube, 215 μL glycine/hydrochloric acid (pH 2.2) buffer solution, 2.5 μL KI (50 mM), 10 μL different concentrations of MTs were added at one time and mixed. Subsequently, 2.5 μL KIO₃ (2 mM) and 50 μL of Au NRs (1.4 nM) were added in turn and placed in a water bath at 50 °C for 5 min after mixing.

3. Results and discussion

3.1. The realization of catalytic activity-modulated etching of CAEPAu with benchmarked colorimetric sensing characteristic

A catalytic plasmonic seed was synthesized from salt citrate-coated Au NPs with a diameter of 22.7 ± 3.7 nm and a UV–vis absorption peak at 518 nm (Fig. 1c and e). The CTAB-coated Au NRs with an aspect ratio of 3:1 with an absorption at 750 nm were synthesized to sense the MTs with visible color changes (Fig. 1d, e). Fig. 1f shows the catalytic reaction process, in addition, the MTs can scavenge reactive hydrogen free radicals generated by NaBH₄ for cysteine’s redox activity, which further restrain the etching of Au NR. Such synergistic effects of MTs corona and reduction properties inhibit Au NPs catalytic activity. The p-NP catalyzed reduction process of plasmonic Au NPs is traced using a UV–vis absorption spectrophotometer. The p-NP solution in the control group presents a colorless and transparent view with a UV–vis absorption peak at 317 nm (Fig. 1g: I). The addition of NaBH₄ changes the solution into alkaline state, thus transforming p-NP into a yellow ionic state with strong absorption at 400 nm (Fig. 1g: II). By adding the bare Au NPs, the reduction process is catalyzed by p-AP, where the absorption at 400 nm fades and the absorption at 300 nm emerges (Fig. 1g: III) with the solution color changing to light pink (Fig. 1g: III).

The key to realize the catalytic activity modulated etching of Au NRs, is to utilize the MTs to inhibit the catalysis of Au NPs. This leads to the early termination of the p-NP catalyst reduction reaction by the protein corona. Results indicate that the absorption at 400 nm decreases but does not fully disappear with orange-yellow solution, indicating that MTs inhibit the catalytic process to a certain extent (Fig. 1g: IV). We previously reported that p-AP produced during the catalytic process will undergo a redox reaction with KIO₃ to generate I₂ [37], which will further etch Au NRs. As a result, it is anticipated that the MTs will inhibit the catalytic effect of Au NPs and weaken the etching of Au NRs by I₂. When adding a small amount of MTs at 0 nM, 3 nM, and 5 nM, the peaks of Au NRs gradually shifted to red, resulting in a transitional change of colors (pink/purplish red/cyan), suggesting that MTs have an inhibitory effect on Au NRs etching by modulating Au NPs catalytic activity (Fig. 1h). Etching of Au NRs can be an interesting method of detecting
MTs visually.

The MTs detection performance of CAEPAu, largely depends on the catalytic reaction parameters (NaBH₄, p-NP, Au NPs, pH, pre-incubation time, and catalytic time) and the etching reaction parameters (pH, etching time, and temperature, KIO₂, Br⁻, and CTAB). The absorbance of p-NP at 400 nm (\(\Delta A, \Delta A = A_{1}-A_{0}\)) is used as an indicator to measure the reaction process. The preliminary experimental results (Fig. S2) suggest that the optimal system is achieved with 0.1 M NaBH₄, 1 mM p-NP, 30 \(\mu\)L Au NPs at 78 pM. Other factors for the catalytic reaction have been benchmarked with a pH of 5 (Fig. S2d), a pre-incubation time of 10 min (Fig. S2e) and a catalytic reaction duration of 5 min (Fig. S2f). As a result of the etching reaction, the peak shift (\(\Delta\lambda_{\text{max}}\)) of ISPR and the color change of Au NRs solution are observed and utilized as indicators of the reaction process and MTs sensing. Similarly, the system settings are optimized with a pH of 2 (Fig. S3a and S4), a reaction time of 20 min (Fig. S3b), and a temperature of 50 °C (Fig. S3c). The optimal concentration of KIO₂ is 50 mM, which can affect the production of sufficient I₂. It is worth noting that Br⁻ and CTAB will stimulate Au NRs etching via an auxiliary etching effect [38], and their optimal concentrations are selected at 50 mM and 25 mM, respectively (Fig. S3d-f).

3.2. The high performance colorimetric sensing of MTs from CAEPAu assay

Visualization-based sensitive detections of MTs are conducted under the above-mentioned benchmarked conditions (Fig. S5). A rigorous ultra-sensitive detection of MTs is unveiled for the obtained CAEPAu assay with strong absorbance at a low concentration range (0–9.0 nM, Fig. 2a), and a signature detection with a MTs concentration of 28 pM which is at least one magnitude higher in sensitivity than any reported method (Table S1). A clear change in Au NRs absorption is also justified with a gradually red-shift of peak (557 nm-743 nm) with the increase of MTs. It is worth mentioning that the \(\Delta\lambda_{\text{max}}\) shows explicit linear relationship at two designated concentration ranges with one at 0–3.5 nM and another at 4.0–6.0 nM (Fig. 2b). Moreover, our CAEPAu assay achieves a rich color spectrum with eight variations (pink, fuchsia, purple, indigo, blue, blue-green, brown, and teal, Fig. 2g), corresponding to a variety of concentrations of MTs in the range of 0–9.0 nM, where the conventional colorimetric assay (Fig. 2h) usually presents indistinguishable visual responses. TEM images show the morphology of Au NRs that are etching at MTs concentrations of 0, 3, 4 and 5 nM (Fig. 2c–f). An explicit variation in the aspect ratio of Au NRs is shown with corresponding color changes at each MTs concentration (pink, purple, indigo, blue-green), which consolidates an underlying structure–property relationship to the CAEPAu strategy. The characterization of particle distribution reveals that the length of Au NRs gradually changes from 19.61 nm to 49.13 nm (Fig. S6) and induce a color change over a broad distribution of MTs concentrations (0–5.0 nM).

After scoping the existing literature to the best of our knowledge [39,40], there is no detection technology with similar capability to detect MTs at such low concentrations (0–9.0 nM) as our CAEPAu colorimetric assay performs. The MTs are cysteine-rich low-molecular weight proteins containing many sulphhydryl groups which can be readily oxidized to make the solution more colorful. We then test the MTs by scavenging oxidizing substances of I₂ (Fig. S5b). When increasing the concentration of MTs, the blue solution generated by iodine, and the starch indicator becomes lighter (Fig. S7). As shown in Fig. S5, the strongest absorption of Au NRs red-shifts gradually (560 nm-723 nm) as the MTs concentration increases (20–400 nM), the \(\Delta\lambda_{\text{max}}\) shows a linear relationship with the concentration of MTs in the range of 20–200 nM. As such, we achieve a rich set of colors responding to extended MTs concentrations (0–400 nM).

We next assess the sensing robustness of CAEPAu colorimetric assay with various interfering chemicals/substances (Fig. S8) such as anions (including 350 nM Cl⁻, CO₃²⁻, NO₃⁻, SO₄²⁻ and H₂PO₄⁻), cations (including 350 nM Na⁺, Mg²⁺, K⁺, Ca²⁺, Cu²⁺ and Fe³⁺), neutral biomolecules (including 350 nM Glu, Urea, Cys and GSH) and proteins (35 nM IgG, HAS, Cyt-c and BSA). CAEPAu assay sensing performance is robust as the impact of common anions and cations is negligible. As a consequence of this interference, a smaller spatial size of bio-thiol is difficult to form on the surface of Au NPs and does not affect their catalytic activity [41,42], which can be mitigated by making the bio-thiol smaller. For smaller MTs, corona action can rapidly mask the catalytic surface of Au NPs, which can be mitigated by making the bio-thiol smaller. For smaller MTs, corona action can rapidly mask the catalytic surface of Au NPs, therefore, the interference from other protein molecules appears to be negligible. The effectiveness of our colorimetric assay is further verified by adding different concentrations to urine, serum, and PBS samples. A spiked recovery of 99.1 %–102.7 % in PBS buffer, 102.5–103.7 % in urine samples, and 105.6–106.0 % in serum is obtained (Table S2), which falls within the reliable detection range and proves the suitability of using CAEPAu colorimetric assay to test samples above.

3.3. The demonstration of using CAEPAu colorimetric assay to detect MTs in tumor cells

We next demonstrate the potential application of this CAEPAu
colorimetric assay to detect MTs in clinic tumor cell samples. A lysate of tumor cells is used (Fig. 3a), with commercial ELISA kits used as a reference/control group (Fig. S9). For the quantitative detection of MTs in cells, the testing result for the reference sample suggests a linear relationship of $y = 0.0360 + 0.3168x$ ($R^2 = 0.9907$) (Fig. S10). The MTs concentration in normal human serum is less than 0.3333 nM (2 ng/mL) [43]. By utilizing normal macrophage cells (RAW264.7) as a control group, MTs of 0.3062 nM and 0.3119 nM are detected for RAW264.7 at different concentrations (Fig. S11, Fig. 3c: I and II), where these results are comparable to those from the commercial ELISA kit. We then applied both assays to determine the abnormal expression levels of MTs in three breast cancer cell samples (MCF-10A, MCF-7, MDA-MB-231) (Fig. 3b: III-VIII and Fig. 3c-e). Interestingly, our CAEPAu colorimetric assay presented sharp and highly distinguishable color changes to reveal the MT concentrations. In contrast, the ELISA results indicate very limited color changes. The examination also extends to three other tumor cells (gastric cancer cells: Sgc-7901, lung cancer cells: H358, and cervical cancer cells: Hela) (Fig. 3f-i and Fig. 3g-i). Similarly, our CAEPAu are more effective than ELISA. The analysis for all cases indicates that the amount of MTs detected by our CAEPAu colorimetric assay is almost the same (∼5%) with that detected by ELISA (Table S3). It is evident from this demonstration that CAEPAu can be demonstrated effective in practical testing of clinic cell samples.

A proof of concept of using CAEPAu colorimetric assay to detect MTs in clinic blood samples from cancer patients. The testing on blood samples has been considered as an effective therapeutic solution to diagnosis cancer with minimal suffering for the patient, as the MTs levels in serum are generally well correlated with the occurrence, development, regression and recurrence of malignant tumor [44]. Since the traditional testing needs wait for the results from the pathology lab for weeks and months, the determination of MTs levels in serum is expected to offer a fast and reliable route to information about malignant tumor diagnosis, efficacy and prognosis. We further quantify the sensing of MTs in clinical whole blood samples collected from eight cancer patients (two breast cancer patients, two cervical cancer patients, two lung cancer patients and two stomach cancer patients). Whole blood samples of clinical patients are centrifuged and separated to obtain serum (Fig. 4a). The results from CAEPAu assay present a dramatic change of color at the MT range of 0.2 to 5.0 nM (Fig. 4b), while the testing results with ELISA only show an incremental gradient change in the same color (Fig. 4c) at a MT range of 0.2 to 9 nM.

Comparisons are also made to assess its practicality and accuracy. MTs detection by this assay on clinical whole blood samples of breast cancer, cervical cancer, lung cancer and gastric cancer can be observed in Fig. 4d-g. The results in the patient’s serum samples show a high consistency with those of the corresponding cancer cells mentioned above (Fig. 4h). The MTs concentration can be determined by analyzing the LSPR peak shift of Au NRs, which is in a good agreement with ELISA results (Table S4). From the above, it is well evidenced that the serum MTs concentration was abnormal in almost all patients.

By summarizing the results from CAEPAu colorimetric assay and the commercial ELISA kit, two colorimetric cards can be wrapped for visual detection of MTs in clinic blood samples (Fig. 4b, c). CAEPAu colorimetric assay shows a relatively small deviation of 0.0174–0.1939 nM from Table S3. In contrast, the ELISA kit shows a deviation of 0.0364–1.6606 nM. The CAEPAu colorimetric strategy presents higher sensitivity.

Another exciting feature of this CAEPAu strategy is its significant time efficient (only 35 min) to process the colorimetric test for each run, compared to the time needed for commercial ELISA kits (3 h for processing each run as standard). Such short processing time of CAEPAu compared to the time needed for commercial ELISA kits (3 h for processing each run as standard) will dramatically improve the diagnosis efficiency and bring huge impact to the healthcare sector. Passing-Bablok regression analysis revealed a slope of 0.778 (95% confidence interval, 0 to 2.33) and an ordinate intercept of 0.544 (95% CI, −3.97 to 2.80), showing an excellent agreement between two methods ($P = 0.63$, Fig. 4i). In addition, the Bland-Altman plot indicates that there are no points outside the 95% limits of agreement, suggesting excellent agreement between two methods (Fig. 4j).
3.4. The universal detection on various proteins and connection with digital interface using CAEPAu colorimetric assay

We further explore the feasibility of using this CAEPAu colorimetric assay on other types of tumor cells. Three proteins, RNAse A [45], GGT [46] and HE4 [47], are selected as typical biomarkers for pancreatic cancer, colorectal cancer and endometrial cancer diseases, respectively. According to RNAse’s specific results, UV–vis data show a maximum shift from 0 to 60 nM (Fig. S12 a, b). Fig. S12 c, d demonstrated a linear relationship between $\Delta \lambda_{\text{max}}$ and RNAse A in the range of 0–25 nM and 30–50 nM, with linear equations of $y = 193.33 - 0.72x (R^2 = 0.9977) $ and $y = 410.2 - 7.9x (R^2 = 0.9924)$. The testing results for GGT and HE4 test are shown in Fig. S12 e–l. Accordingly, the RNAse A, GGT and HE4 colorimetric cards obtained by this CAEPAu colorimetric assay can be explicitly identified with the naked eye at concentrations ranging from 0 to 60 nM, 3.4–6.5 nM and 0–2.5 nM, respectively (Fig. S12-I–III). To provide a reliable connection to the digital interface, RGB readings from the colorimetric cards of four marker assays are extracted, as shown in Fig. 5a: l–IV (MTs, RNAse A, GGT and HE4) and Fig. S13a–d. After evaluating the results in Fig. 5a: V (admixture) and 5b, we can see a clear difference between the composite swatches obtained by adding 2 nM of MTs to RNAse A, GGT, and HE4 expression levels. It should be emphasized that the presence of universal tumor markers MTs does not compromise the judgment of these markers in this mixed state. On the contrary, the color signal has been enhanced by diverse and broad color differentiation. This provides a direct and visible way to identify tumor by color expression. An analysis of the color spectrum of CAEPAu and ELISA is carried out by plotting the signature color range from the two sensing probes (Fig. 5c and Fig. S13e–h), we can see that CAEPAu has a wider distribution than ELISA, providing a comprehensive mechanism to detect and recognize MTs at various concentrations, with excellent discrimination.

4. Conclusions

In summary, we described a facile colorimetric strategy for early-
stage tumor diagnosis by detecting MTs at an ultra-low concentration range (0–9 nM). The protein corona effect of MTs that modulates the catalytic activity of Au NPs and thus controllably regulates the extent of etching of Au NRs, to realize a colorimetric assay with precise response to the MTs which can be visualized by a naked-eye. The colorimetric assay shows excellent linearity in 0.1–3.5 nM with an ultra-sensitivity as low as 28 pM. It possesses remarkable superiority to commercial kits in detection time with taking only 12 % of the testing time for commercial ELISA kit. This CAEPAu colorimetric strategy has been proved effective on a number of tumor cells (breast cancer cells, lung cancer cells, cervical cancer cells, etc.), clinic blood samples and other proteins, therefore has great potential in the therapeutic application to diagnosis cancer at early-stage.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Caiping Ding designed the idea and wrote the manuscript, Ming Li conceived the idea and designed the experiments. Ming Li, Weiwei Chen and Zikang Chen analyzed the data. Zhanhu Guo, Zhiyong Guo and Xuehua Zhang co-revised the paper. Ben Bin Xu and Youju Huang supervised the whole project.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supporting Information. UV-vis absorption spectra of p-NP and p-AP, Optimization of catalytic reaction and etching reaction conditions, UV-vis absorption spectra changes before and after scavenging of superoxide radicals by MTs, Table of comparison studies for the detection of MTs with some previously reported work, selectivity of MTs detection, Table of analytical performance of this sensor in detecting MTs in PBS, human urine and serum, standard sample analysis of the kit, detection of MTs in macrophages, Table of comparison of test results of MTs concentration in tumor cell samples, standard colorimetric cards for the detection of RNAse A, GGT and HE4. Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2023.146689.

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