Gold-Loaded Nanoporous Ferric Oxide Nanocubes with Peroxidase-Mimicking Activity for Electrocatalytic and Colorimetric Detection of Autoantibody


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ABSTRACT: The enzyme-mimicking activity of iron oxide based nanostructures has provided a significant advantage in developing advanced molecular sensors for biomedical and environmental applications. Herein, we introduce the horseradish peroxidase (HRP)-like activity of gold-loaded nanoporous ferric oxide nanocubes (Au−NPFe2O3NC) for the development of a molecular sensor with enhanced electrocatalytic and colorimetric (naked eye) detection of autoantibodies. The results showed that Au−NPFe2O3NC exhibits enhanced peroxidase-like activity toward the catalytic oxidation of 3,3′,5,5′-tertamethylbenzidine (TMB) in the presence of H2O2 at room temperature (25 °C) and follows the typical Michaelis–Menten kinetics. The autoantibody sensor based on this intrinsic property of Au−NPFe2O3NC resulted in excellent detection sensitivity [limit of detection (LOD) = 0.08 U/mL] and reproducibility [percent relative standard deviation (% RSD) = <5% for n = 3] for analyzing p53-specific autoantibodies using electrochemical and colorimetric (naked eye) readouts. The clinical applicability of the sensor has been tested in detecting p53-specific autoantibodies in plasma obtained from patients with epithelial ovarian cancer high-grade serous subtype (EOCHGS, number of samples = 2) and controls (benign, number of samples = 2). As Au−NPFe2O3NC possess high peroxidase-like activity for the oxidation of TMB in the presence of H2O2 [TMB is a common chromogenic substrate for
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Iron oxide (IO) nanostructures have widely been used in a variety of biomedical applications, such as tissue engineering, magnetic resonance imaging, hyperthermia, drug and gene targeting, isolation and separation of proteins or cells from samples, and in vivo cell tracking. The paramagnetic properties of IO nanoparticles also allow for contactless sample preparation and handling using the emerging technology of micromagneto-fluidics. In recent years, it has been demonstrated that IO-based nanoparticles (NPs) possess an intrinsic horseradish peroxidase (HRP)-mimicking activity toward the oxidation of common chromogenic substances such as 3,3′,5,5’-tetramethylbenzidine (TMB), diazoaminobenzene, and o-phenylenediamine, which have widely been used in catalytic decomposition of hydrogen peroxides or developing nonenzymatic glucose sensors. For instance, the peroxidase-like activity of FeOx, Prussian blue/FeOy, graphene/FeOy, GO-MNP(FeOy)/Pt, and ZnFe2O3-NPs has extensively been used to develop several high-performance biosensors for detecting glucose, H2O2, cancer cells, l-cysteine, etc. Similar to IO-based NPs, other nanomaterials such as AuNPs, EuOx-SNPs, CuONPs, copper-creatinine complex, AgX (X = Cl, Br, I), polyoxometalates, PtNPs, and carbon nanotubes and nanodots have also been reported to exhibit peroxidase-like activity. The use of these peroxidase-mimetic nanomaterials for developing biosensors is highly attractive due to several reasons. First, unlike natural peroxidase (i.e., HRP) they are stable toward protease digestion or denaturation, and their structure, morphology, and function are not affected by environmental stresses. Second, they are relatively easy and inexpensive to prepare and highly suitable for benchtop storage and handling. Despite these advantages, most of these materials demonstrated their highest peroxidase-like activity at higher temperature (40–45 °C), which limits their applications in disease-specific biomolecules detection at room temperature. Therefore, design and synthesis of NPs with enhanced peroxidase-mimetics activity at room temperature are highly desirable.

Recently, we have synthesized a new class of gold-loaded super-paramagnetic ferric oxide nanocubes (Au–NPFe2O3NC). This Au–NPFe2O3NC exhibits multiple functionalities. First, it shows enhanced catalytic activity toward the common electroactive molecules [i.e., hexaamin erotenium(III) chloride, (Ru(NH3)6Cl3)]23. Second, the highly porous framework of gold-loaded FeOx nanocubes allows for the direct adsorption of a large amount of target DNA, RNA, or protein via gold–DNA/RNA/proteins affinity interactions. Third, as these nanocubes are paramagnetic, they can be used as dispersible capture vehicles to isolate the circulating biomarkers in body fluids (i.e., they can be dispersed into the sample and bind to the analytes of interest). As the capture event of the target analytes of interest is temporally and spatially separated from the electrochemical measurement, this alleviates biofouling issues of the electrode. The Au–NPFe2O3NC also provide a means to reduce the biological noises associated with adsorption of non-specific species present in the body fluids via magnetic enrichment, separation, and purification steps. Fourth, the Au–NPFe2O3NC can also be used as nanoenymes with enhanced peroxidase-like activity. Although the functionalities of Au–NPFe2O3NC as electrocatalyst and dispersible capture vehicle have already been explored, the peroxidase-like activity of Au–NPFe2O3NC has yet to be demonstrated. In this paper, we studied this peroxidase-like activity for the development of a proof-of-concept molecular sensor for detecting circulating autoantibodies in serum or plasma samples.

Circulating autoantibodies that are elicited in responses to tumor-associated antigen (TAA) are emerging biomarkers for the early detection of cancer, as they are produced by the patient’s immune system long (several months or years) before the onset of the clinical symptoms of diseases. The direct quantification of TAAAs in clinical samples possesses severe challenges due to their low abundance and associated difficulties in identifying minor structural modification or mutation. In contrast, serum autoantibodies are relatively more stable (i.e., longer half-lives due to limited proteolysis and clearance) and present as large quantities in clinical samples, and therefore, they have been used as circulating reporters for the early or preclinical detection of various cancers including ovarian, lung, and breast cancer. It is also important to note that, with the progression of the cancer (stages), the amount of autoantibody (produced) compared to that of TAAAs is highly significant and readily detectable with the conventional detection techniques.

Most of the widely used methods for serum autoantibody detection are mainly based on enzyme-linked immunosorbent assays (ELISA) or protein arrays that employ HRP-conjugated protein-specific secondary antibodies to read the target autoantibody. These methods are, however, expensive, relatively less sensitive, and can only provide qualitative or semiquantitative results. Until recently, the most advanced assay for the sensitive detection of serum autoantibody is that developed by Garranzo-Asensio et al., where HaloTag fusion p53 protein modified commercial magnetic beads (MBs) were used as magnetic microcarriers for capturing the autoantibodies in sera. HRP-conjugated anti-human IgG was then used to quantify captured autoantibody via colorimetry and amperometry. More recently, we have also developed a method using Au–NPFe2O3NC as a dispersible capture agent for autoantibody isolation and detection. Although the analytical performances of these methods are superior, they still rely on HRP-based enzymatic reaction.

Herein, we first studied the peroxidase-like activity of Au–NPFe2O3NC toward the catalytic oxidation of TMB in the presence of H2O2. This feature of Au–NPFe2O3NC was then used to develop a molecular sensor for the colorimetric (naked eye) and electrochemical detection of p53 autoantibody in serum or plasma samples. The method was first tested on the sample obtained from commercial p53 ELISA kit and finally challenged with a small cohort of plasma samples derived from epithelial ovarian cancer high-grade serous subtype (EOCHGS).

**EXPERIMENTAL SECTION**

**Study Group and Ovarian Cancer Samples.** Staged samples (cross-sectional) were collected at the Ochsner Baptist Medical Center in the clinical trials and obtained via The UQ Centre for Clinical research. Plasma samples were obtained in accordance with the declaration of Helsinki and approved by the Ethics Committee of The University of Queensland and the Ochsner Medical Center (New Orleans, LA, U.S.A.). Plasma was separated from whole blood by centrifugation (2000 × 10 min at room temperature) and stored at −80 °C until analyses.
Ovarian cancer samples were collected prospectively and assigned according to the hystotypes classification (e.g., high-grade serous subtype; stage I and stage III) and stored to −80 °C in the Biobank units. Only patients with epithelial ovarian cancer high-grade serous subtype (n = 2) and benign controls (n = 2) were included in this study.

Peroxidase-Mimetic Activity of Au−NPFe2O3NC. Unless otherwise stated, the peroxidase-like activity of Au−NPFe2O3NC was carried out at room temperature using 5 μg of Au−NPFe2O3NC in 80 μL of reaction buffer (0.2 M sodium acetate (NaAc), pH 3.5) in the presence of 800 μM freshly prepared TMB (TMB dissolved in DMSO) and 700 mM H2O2. The formation of blue-colored solution was monitored and measured in time scan mode at 652 nm using a spectrophotometer (SpectraMax). The reaction was quenched by adding 2.0 μL of stop solution. The end point of the resultant yellow color product was measured using colorimetry (at 452 nm) and amperometric readouts (SPE-Au) and amperometric response (i−t) was measured at 150 mV over 120 s. At least three replicates were measured for each standard/sample. All measurements were performed at room temperature.

RESULTS AND DISCUSSION
Peroxidase-Mimetic Activity of Au−NPFe2O3NC. It is now well-established that natural peroxidase (HRP) can catalyze the oxidation of TMB in the presence of H2O2. This catalytic reaction generates two colored products. First, HRP/H2O2 catalyzed the oxidation of TMB that produced a blue-colored charge-transfer complex of parent TMB (diamine) and TMB oxidized product (dimine), which could be used for qualitative (naked eye) evaluation. Second, this blue-colored complex turned yellow after the addition of an acid.4,41 The yellow product is electroactive and stable at acidic pH and, thus, can be quantified by UV−vis (semiquantitative, colorimetry) and electrochemical detection methods. We have recently synthesized a novel class of superparamagnetic Au−NPFe2O3NC.21,22 The details characterization data of this material have already been described in refs 21 and 22. Briefly, Au−NPFe2O3NC was synthesized by depositing AuNPs onto the nanoporous iron oxide nanocubes (NPFe2O3NCs) derived from Prussian blue (PB) nanocubes.21,22 Bright-field transmission electron microscopy (TEM) and high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) images for this materials clearly showed that they possessed highly porous structure (Figure S1 in the Supporting Information). Scanning electron microscopy (SEM) images in Figure S2 (Supporting Information) resulted in that the Au content is well-distributed over the entire particle. The peaks in the wide-angle X-ray diffraction (XRD) pattern are assignable to Au, α-Fe2O3, and γ-Fe2O3 (Figure S3 in the Supporting Information).48 This material exhibits peroxidase-like activity for the oxidation TMB in the presence of H2O2, at room temperature. Figure 1a represents the mechanism of the catalysis of TMB (in the presence of H2O2) oxidation reaction by Au−NPFe2O3NC.

To assess peroxidase mimetics of nanocubes, a set of control experiments were conducted. TMB substrate solution in the presence of H2O2 was added and incubated for 30 min in the dark. The color change was visually observed. For quantitative measurements of the amount of oxidized TMB, 2.0 μL of stop solution (2.0 M HCl) was added and absorbance was recorded at 452 nm with the spectrophotometer. For the electrochemical detection, 30 μL of the oxidized TMB solution was pipetted onto another clean screen-printed gold electrode (SPE-Au) and amperometric response (i−t) was measured at 150 mV over 120 s.

Colorimetric and Amperometric Detection of Serum p53 Autoantibody. The neuravidin-modified screen-printed carbon electrode (SPCE-XTR) was kept at room temperature for 1 h and washed with phosphate buffer solution (PBS). An amount of 5.0 μL (100 ng/mL) of p53 antigen was incubated onto the electrode surface for 40 min. After the incubation, the electrode surface was washed with PBS three times followed by further incubation with 1% bovine serum albumin (BSA) solution (blocking agent) for 15 min. An amount of 5.0 μL of diluted serum sample or tested serum sample (1:100 dilution) was then added to the electrode surface and incubated for 1 h to capture the p53-specific autoantibodies present in the sample. After washing away the unbound or loosely attached serum proteins with PBS, the complex is incubated with 5.0 μL of freshly prepared IgG/Au−NPFe2O3NC for 40 min followed by several PBS washes to remove all unbound IgG/Au−NPFe2O3NC.
activity of our novel Au–NPFe2O3NC. Notably, unlike most of the existing nanomaterials \(^1\)–\(^{13}\) which possess the highest peroxidase-like activity at 40–45 °C, our Au–NPFe2O3NC exhibit relatively enhanced activity at room temperature. The level of peroxidase-like activity of Au–NPFe2O3NC at room temperature is sufficient enough to generate approximately 20 times higher response in comparison with the negative control sample (Figure 1c). The possible explanation of this enhanced response could be due to (i) the large surface of the nanocubes-catalyzed TMB/H2O2 reaction, ferric ions (from eq 8.44) and produce an excessive amount of FeOOH\(^2+\) (see above) and (ii) the presence of 2% AuNPs in the nanocubes that also catalyze the TMB/H2O2 reaction. In this nanocubes-catalyzed TMB/H2O2 reaction, ferric ions (from nanocubes) initiate the reaction by generating hydroxyl free radical (-OH) from H2O2 following the Fenton reaction.\(^{42,43}\) TMB is then oxidized by the generated -OH, as shown below:

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\begin{align*}
\text{Fe}^{3+} + \text{H}_2\text{O}_2 & \rightarrow \text{FeOOH}^{2+} + \text{H}^+ \quad (4) \\
\text{FeOOH}^{2+} & \rightarrow \text{Fe}^{2+} + \text{HO}_2^- \quad (5) \\
\text{Fe}^{2+} + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH} \quad (6) \\
\cdot\text{OH} + \text{TMB} & \rightarrow \text{TMB}_{\text{ox}}\text{(blue)} \quad (7)
\end{align*}
\]

Similar to HRP, the activity of Au–NPFe2O3NC is also dependent on the solution pH and amount of nanocubes. In order to check the effect of pH, we studied the peroxidase-like activity of nanocubes using different solution pH values ranging from 2.5 to 5.5. We observed that with increasing pH of the solution the peroxidase-like activity of Au–NPFe2O3NC decreases (Figure S4a1 in the Supporting Information). This is because of the coexistence of negatively charged TMB and hence the presence of 2% AuNPs in the nanocubes in our assay, 5 \(\mu\)L of designated concentrations [i.e., 2.5 (0.5 \(\mu\)g/\(\mu\)L), 5 (0.5 \(\mu\)g/\(\mu\)L), 10 (2 \(\mu\)g/\(\mu\)L), and 20 \(\mu\)g (4 \(\mu\)g/\(\mu\)L)] of nanocubes was used. As can be seen in Figure S4b1 (in the Supporting Information), a similar phenomenon commonly observed from H2O2 (Figure 2a) and TMB (Figure 2b). To obtain the catalytic parameters [Michaelis–Menten constant (\(K_m\)) and maximum velocity (\(V_{\text{max}}\))] for both H2O2 (Figure 2a) and TMB (Figure 2b). To obtain the catalytic parameters [Michaelis–Menten constant (\(K_m\)) and maximum velocity (\(V_{\text{max}}\)), the data were fitted to the Michaelis–Menten kinetic model using a nonlinear least-squares fitting.\(^{39}\) All kinetic parameters were also calculated from the Lineweaver–Burk double-reciprocal plot (1/velocity \([V]_o\) vs 1/substrate concentration \([S]\)) (inset of Figure 2)\(^{40}\) and compared with that for previously reported peroxidase-mimetic nanoparticles (Table S1 in the Supporting Information). \(K_m\) is an indicator of enzyme affinity toward its substrate, and a lower \(K_m\) indicates the stronger affinity between enzymes and substrates. The apparent \(K_m\) value for Au–NPFe2O3NC with TMB was lower than that

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\cdot\text{OH} + \text{HO}_2^-/\text{O}_2^- \rightarrow \text{H}_2\text{O} + \text{O}_2 \quad (9)
\]

Among all studied pH values, pH 2.5 resulted in the highest responses both in colorimetry and amperometric readouts. As the iron could be leached from the nanocubes in the solution pH of <3, we selected pH 3.5 as the optimal pH for conducting subsequent experiments in our study. To optimize the amount of nanocubes in our assay, 5 \(\mu\)L of designated concentrations (Figure S5 in the Supporting Information), a similar phenomenon commonly observed in colorimetry and amperometric readouts, and therefore we chose 5 \(\mu\)g as the optimal nanocube amount for subsequent experiments.

**Steady-State Kinetics for Au–NPFe2O3NC.** To investigate the peroxidase-like activity of nanocubes, apparent steady-state kinetic parameters for TMB oxidation were determined by varying the concentration of H2O2 and TMB (Figure S5 in the Supporting Information), a similar phenomenon commonly observed for HRP enzymes.\(^{7,45}\) The experiments were carried out using 5 \(\mu\)g of nanocubes in a reaction volume of 60 \(\mu\)L (0.2 M NaAc buffer, pH 3.5) at room temperature. The kinetic parameters were estimated by initial rate method.\(^{7,45}\) The absorbance data were converted to corresponding concentration by the Beer–Lambert law using the value of \(\varepsilon = 39,000 \text{ M}^{-1} \text{ cm}^{-1}\) (at 652 nm) for the oxidized product of TMB.\(^{46}\) A typical Michaelis–Menten-like curve was obtained within the suitable concentration range for both H2O2 (Figure 2a) and TMB (Figure 2b). To obtain the catalytic parameters [Michaelis–Menten constant (\(K_m\)) and maximum velocity (\(V_{\text{max}}\))], the data were fitted to the Michaelis–Menten kinetic model using a nonlinear least-squares fitting.\(^{39}\) All kinetic parameters were also calculated from the Lineweaver–Burk double-reciprocal plot (1/velocity \([V]_o\) vs 1/substrate concentration \([S]\)) (inset of Figure 2)\(^{40}\) and compared with that for previously reported peroxidase-mimetic nanoparticles (Table S1 in the Supporting Information). \(K_m\) is an indicator of enzyme affinity toward its substrate, and a lower \(K_m\) indicates the stronger affinity between enzymes and substrates. The apparent \(K_m\) value for Au–NPFe2O3NC with TMB was lower than that.
with HRP, suggesting that the nanocubes have higher affinity with TMB in comparison to the HRP. Moreover, the $K_m$ value with both TMB and H$_2$O$_2$ is also higher than that of nonporous Fe$_3$O$_4$ NPs. The enhanced peroxidase activity of our nanocubes at room temperature can be related to the highly porous ferric oxide moiety with high surface area (exposure of more Fe(III) ions) and large pore volume that facilitates the increased mass transfer as well as enhances the kinetics of the reaction. Additionally, as outlined earlier for PB$^{-}$Fe$_2$O$_3$, the nanoporous structure of Au$^{-}$NPFe$_3$O$_4$NC could be beneficial for increasing electron transfer from the top of the valence bond of Fe$_3$O$_4$ to the lowest unoccupied molecular orbital (LUMO) of H$_2$O$_2$. Moreover, transfer of lone-pairs electron density (charge transfer) from the amino group of TMB to the vacant d orbital of Fe$^{3+}$ may also enhance the electron density and mobility of Au$^{-}$NPFe$_3$O$_4$NC.

**Colorimetric and Amperometric Detection of Autoantibody.** Our assay for the detection of p53-specific autoantibody using peroxidase-mimetic Au$^{-}$NPFe$_3$O$_4$NC is schematically represented in Figure 3. Here, p53 antigen was used to selectively recognize and capture the p53-specific autoantibody present in serum and plasma samples. In this proof-of-concept assay, we have chosen p53 autoantibody, because mutation in TP53 proteins is an early indicator of cancer and also found to be present in almost 80% of all carcinogenesis. In response to the expression and mutation of this protein, the corresponding amounts of p53-tumor antigen-associated autoantibodies (TAAb) are generated, which has been considered as a potential
early diagnosis biomarker for ovarian (type-II) cancer. In our assay, first, a neuravidin-modified screen-printed carbon electrode (SPCE) was modified with biotinylated p53 antigen through standard biotin–avidin chemistry. Bovine serum albumin was then used to block the unreacted sites to reduce nonspecific bindings of biomolecules followed by the incubation of serum or plasma samples containing target p53 autoantibody. As protein has the strong affinity toward a gold surface, we functionalized Au–NPFe₂O₃NC with α-human IgG to form IgG/Au–NPFe₂O₃NC nanocatalysts. These nanocatalysts were then added to the electrode surface to form an immunocomplex with the target p53 autoantibody. To achieve the readout signals, the electrode surface was incubated with the freshly prepared TMB/H₂O₂ solution. The nanocatalysts initiate the oxidation of TMB, thereby producing a blue-colored charge-transfer complex (one electron). After the addition of stop solution, the blue-colored product was converted to a stable, electroactive yellow-colored (diimine) product. The naked-eye observation of the generation of blue and subsequent yellow colors demonstrated the presence of p53-positive autoantibody present in analytes. The intensity of the TMB₅₂(₅) is directly linked to the amount of nanocubes as well as amount of p53 autoantibody present on the electrode surface. Qualitative evaluation was observed by naked eyes. Colorimetric readout (semiquantitative) was conducted by measuring the absorbance of the diimine product at 452 nm. However, the diimine product is electroactive, which allows the further quantification of autoantibody by chronoamperometry.

To evaluate the assay specificity and functionality, we performed our assay in both p53-positive and p53-negative samples. Diluted serum samples from commercial ELISA kit (ELISA-kit, Dianova GmbH, Germany) containing p53 autoantibody was used as a positive sample and serum samples without p53 autoantibody were taken as negative samples. The p53 autoantibody concentration in diluted human plasma sample is 14 U/mL, where 1.0 U represents the p53 binding activity of 100 μL of undiluted calibrator. We found that positive samples containing 7.0 U/mL (1:1 dilution) of p53 autoantibody gave an intense blue color, while the stoichiometric amount of negative samples remained colorless. As can be seen in Figure 4a, a 10 times higher absorbance (absorbance at 452 nm) was observed for the positive sample compared to that of the negative sample (0.616 vs 0.0526). Chronoamperometric measurement of the positive sample was found to be 16 times higher than that of the negative one (36 vs 2.116 μA cm⁻²) (Figure 4b). Control experiment without IgG/Au–NPFe₂O₃NC nanocatalysts gave negligible responses in both absorbance (absorbance at 452 nm = 0.0526) and subsequent amperometric (1.9 μA cm⁻²) measurements. This can be explained by the fact that, in the absence of nanocatalysts, TMB (in the presence of H₂O₂) oxidation reaction does not occur thereby resulting in negligible response. In another control experiment, we used only phosphate buffer instead of serum with p53 autoantibody sample. This experiment resulted in a slightly higher response than that of the control without nanocatalysts (absorbance at 452 nm, 0.0625 vs 0.0526, and current 3.63 vs 1.9 μA cm⁻²). This may be due to a level of nonspecific interaction of nanocatalysts with surface attached p53 antigen. All these control experiments clearly demonstrated the good level of specificity of our assay toward the detection of p53 autoantibody from serum samples with negligible background response.

To evaluate the sensitivity of our assay, a series of diluted positive samples obtained via serial dilution (from 1:1 to 1:80; 7.0–0.0875 U/mL) was tested. We observed an increasing trend in both the absorbance and current response with the increasing concentration of target autoantibody (Figure 4). This is because the higher amount of target p53 autoantibody could bind an increasing amount of nanocatalysts on the electrode surface, which subsequently enhance the catalytic activity of TMB (in the presence of H₂O₂) system. As shown in Figure 5a, the colorimetric responses increase linearly with the increasing concentration of serum samples, and the linear regression equation was estimated to be y = 0.04741x − 0.0226, with the correlation coefficient (r²) of 0.9684. The detection limit (LOD) was estimated by corresponding signal-to-noise ratio of 2.5, and was found to be 0.12 U/mL for the colorimetric readout. On the other hand, the linear regression equation for amperometric readout was estimated as y = 5.201x − 1.4849, with the r² of 0.9961, confirming the relatively better sensitivity (LOD of 0.08 U/mL, Figure 5b) in comparison to the colorimetric readout. A relative standard deviation (% RSD) of three different sensors for both the colorimetric and electrochemical readouts was estimated to be <5.0%, suggesting the good reproducibility of electrode surface modification, isolation of p53 autoantibody from serum samples, incubation of nanocatalysts onto the p53 autoantibody-attached electrode surface, and nanocatalysts-induced signal transduction protocols. This level of LOD and reproducibility of our colorimetric and electrochemical

**Figure 4.** Mean responses of (a) absorbance and (b) steady-state amperometric current obtained for the assay with one positive (presence of p53 autoantibodies in serum) and with three negative control samples (no target represents PBS instead of positive serum, negative control represents serum without p53 autoantibodies, and no secondary antibody represents the no IgG/Au–NPFe₂O₃NC). Insets: corresponding photos for the naked-eye evaluation and i–t curves.
assay over a wide range of serum concentrations clearly demonstrates that the peroxidase-like activity of Au−NPFe2O3NC nanocatalysts is sensitive and specific enough to detect autoantibody in p53-positive serum samples. Moreover, the LODs for both readout systems are better than that of the conventional p53-ELISA kit (0.08 vs. ~0.3 U/mL).52 These LODs are also better than that of a recently reported Halotag fusion protein modification based electrochemical platform (0.08 vs 0.34 U/mL).38 Therefore, our methods can detect much lower concentration of p53 autoantibody than the above-reported methods. It is also important to note that the sensitivity of our recently reported HRP-based method is slightly better than that of the current method (i.e., 0.02 vs 0.08 U/mL). However, our current method avoids the use of an expensive HRP-based reaction system.

We further checked the applicability of our novel platform for the analysis of complex human plasma samples. The plasma samples were obtained from women with epithelial ovarian cancer high-grade serous subtype (stages I and III, P3 and P4, respectively) and controls (P1 and P2) (benign). Ovarian cancer is one of the leading causes of cancer-related death of woman from gynecologic malignancy.9 It has been reported that, in response to the overexpression or mutation of protein (TP53) in ovarian cancers, p53-antigen-specific autoantibodies are generated.35 TP53 mutation occurs early in high-grade ovarian cancer and is strongly attendant with the p53 autoantibodies.35 Thus, development of an early diagnosis platform for ovarian cancers via p53 autoantibody analysis can potentially decrease the disease burden as well as increase the overall survival. In this study, all clinical samples were diluted (1:100) prior to performing the assay. As projected, both colorimetric and electrochemical methods responses were higher in the stage III patient’s (P4) sample than stage I (P3) (Figure 6). However, the assay can also differentiate the stage I response from the two noncancerous healthy controls. Both of these healthy controls (P1 and P2) produced negligible signal suggesting the absence of p53 autoantibody. These clinical data showed a very good interassay reproducibility (RSD < 5%, for n = 3) for the analysis of differential expression pattern of p53 autoantibodies in different stages of ovarian cancers.

The naked-eye discrimination of the autoantibodies described here holds huge potential for the development of a user-friendly and inexpensive bioassay in resource-limited settings, where sophisticated scientific equipment is unavailable. In particular, this approach can be exploited as a rapid first-pass screening (yes/no) tool to detect clinically relevant autoantibodies in a large population followed by more accurate and sensitive quantification of autoantibody via amperometric readout. The use of a disposable SPE-Au successfully eliminates the need of a time-consuming electrode cleaning process typically used in

Figure 5. Concentration-dependent curve for p53 autoantibody standards provided in the commercial p53 autoantibody ELISA kit. Mean responses of (a) absorbance and (b) steady-state amperometric currents obtained for the designated concentration of standard samples. The inset shows the corresponding linear regression curves.

Figure 6. Mean responses of (a) absorbance and (b) steady-state amperometric current obtained p53-specific autoantibodies present in plasma samples obtained from patients with epithelial ovarian cancer high-grade serous subtype (EOCHGS, P4 = stage III and P3 = stage I) and noncancerous healthy patients (benign, P1 and P2).
conventional disk electrodes. The assay also replaces natural enzymes for TMB oxidation, and thus reduces the cost, handling, and storage facilities generally required for natural enzymes. Overall, the assay platform is relatively inexpensive and portable (i.e., uses disposable and inexpensive SPE; replaces relatively expensive HRP; avoids tedious cleaning of conventional disk electrodes). Importantly, the application of this method is not limited to autoantibody detection; it could potentially be applied as an ideal alternative for conventional ELISA assays for the detection of many other clinically relevant protein biomarkers by changing the relevant antibodies in the antibody functionalization steps of the assay. Taken together these benefits, we expect that this peroxidase-like activity of Au-NPFe2O3NC and their subsequent translation to p53 autoantibody detection assay may have wide application in human cancers or chronic disease.

**CONCLUSIONS**

We have introduced the peroxidase mimetics of a new class of Au-NPFe2O3NC. We have shown that Au-NPFe2O3NC resulted in the enhanced peroxidase-like activity and followed the Michaelis–Menten and Lineweaver–Burk models for the enzyme-catalyzed TMB/H2O2 reaction at room temperature (25 °C). The enhanced peroxidase-like activity was mainly due to the large surface of the Au-NPFe2O3NC (i.e., highly porous) that facilitated the binding of increased amount of positively charged TMB and hence the TMB/H2O2 reaction. The presence of 2% AuNPs within the nanocube framework also contributed toward the catalysis of the TMB/H2O2 reaction. This intrinsic feature was further used to develop a new proof-of-concept platform for autoantibody detection in body fluids samples using both colorimetric and electrochemical readout. This platform successfully detected the p53 autoantibodies in diluted serum and a small cohort of patients’ plasma samples with high sensitivity and specificity.

**ASSOCIATED CONTENT**

 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b02880.

Comparison of the kinetic parameters of Au-NPFe2O3NC and reported nanomaterials, detailed experimental section, TEM, SEM, and XRD results, optimization of pH and nanocube concentration, and dependence of the UV−vis absorbance of the Au-NPFe2O3NC-catalyzed reaction on H2O2 and TMB concentration (PDF).

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Notes

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by the NHMRC CDF (APP1088966) to M.J.A.S. and HDR scholarships to M.K.M. (from the University of Wollongong), S.Y. and M.N.I. (from Griffith University). Y.Y. and Z.A.A. are grateful to the Deanship of Scientific Research, King Saud University for funding through Vice Deanship of Scientific Research Chairs. Lions Medical Research Foundation (C.S), UQ-Ochsner Seed Fund for Collaborative Research (C.S and R.K), and Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT 1170809) (C.S). The authors would like to thank Dr. Macc Bio-Pharma Private Limited for preparation of iron oxide samples.

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