



Using Nanomaterials in Colorimetric Toxin Detection

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Abstract

Exposure to toxins through contaminated food is a serious concern. For the detection of toxins in complex matrices, there are many analytical instrumentation-based methods; however, these approaches are generally expensive, laborious to perform, and require skilled technicians. Thus, they can only be utilized in centralized laboratories. To efficiently prevent the contamination by toxins and improve food safety, the use of on-site toxin detection methods enabling simple, rapid, sensitive, specific, reliable, and affordable identification of toxins is required. A colorimetric toxin detection strategy providing a naked-eye readout platform suits these requirements. Notably, the implementation of nanomaterials in the colorimetric strategy has proven to rapidly generate a higher capacity for detectable color responses owing to their unique physicochemical and catalytic properties. In this review, recent research on colorimetric toxin detection utilizing diverse nanostructures including noble metal nanoparticles and enzyme-like catalytic nanomaterials (nanozymes) is reviewed and discussed. Current challenges and future prospects for the utilization of nanomaterials in colorimetric toxin detection are also discussed.

Keywords Colorimetric biosensors · Toxin detection · Point-of-care testing · Noble metal nanoparticle · Nanozyme

1 Introduction

Natural toxins or biotoxins are noxious compounds that are prevalent in natural environments. These are secondary metabolites produced by living organisms such as molds, fungi, bacteria, algae, plants, and other marine species. Although these substances are harmless to the organisms themselves, they may be lethal to other species, including humans [1]. Unlike bacterial toxins, which are mainly proteins, toxins derived from fungi and algae are not proteins and cannot be detected by the human immune system [2]. Additionally, these toxins are rarely destroyed by the conventional heating or cooking processes, making them more virulent and threatening to humans. In accordance with the origin of biotoxins, they can be categorized into three main groups, including fungal, algal, and bacterial toxins. Mycotoxins are fungal toxins and originate from many filamentous fungal species, such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* [3]. These toxins commonly exist in a variety

of foods through many contamination routes, such as the pre- and post-harvesting stages or during processing, transportation, and storage. According to a reported estimation, approximately 25–50% of all merchandise produced worldwide, in particular basic food supplies, is polluted with mycotoxins to some extent [4]. Furthermore, these toxins can be potential mutagens, carcinogens, and teratogens [2]. Toxins produced from algae (algal toxins) are not only considered to be hazardous to humans, but also negatively affect aquatic habitats and ecosystems. Some algae from freshwater and marine habitats are able to produce numerous types of toxins that further accumulate in shellfish or finfish via the food chain and are ultimately consumed by humans. The consumption of these toxins at acute levels leads to clinical symptoms such as paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), and neurotoxic shellfish poisoning (NSP) [5].

Currently, analytical methods, such as solid-phase extraction, surface plasma resonance, high-performance liquid chromatography, liquid chromatography–mass spectrometry (LC–MS), enzyme-linked immunosorbent assay (ELISA), fluorescence, and chemiluminescence methods, have been widely used to detect biotoxins. Nonetheless, these techniques are not only expensive and complicated but also time-consuming, require skilled personnel, and can only

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be adapted in modernized and centralized laboratories [2, 5]. With the necessity of on-site toxin monitoring, a much simpler strategy with sufficient detection performance and cost-effectiveness should be established. Point-of-care testing (POCT) for clinical diagnosis has attracted considerable interest, because it can provide a convenient, real-time, and portable yet effective medical analysis [2]. Many detection strategies, such as electrochemical, fluorescence, chemiluminescence, and colorimetric methods, have been developed for establishing point-of-care biosensors. Among them, the colorimetric strategy is of tremendous interest, because it provides simplicity to construct the biosensor, is cost effective, and yields a visual signal that can be easily distinguished by the naked eye and requires no external detection devices [6]. Furthermore, the utilization of nanomaterials has recently garnered much attention because of its capability to enhance the performance of colorimetric biosensors, resulting in a higher signal outcome (sensitivity) with a lower limit of detection (LOD) [7]. As nanomaterials have a high surface-to-volume ratio, they can be efficiently functionalized with bioreceptors, allowing specific interactions with target toxins [7, 8]. Owing to the nanoscopic size, nanomaterials have distinct chemical and physical features that are beneficial for rapid signal generation and its amplification [9, 10].

In this review, we describe recent research developments in nanomaterial-based colorimetric detection of biotoxins. We describe the representative ways how nanomaterials have been utilized in this field, including noble metal nanoparticle-mediated color transition and nanozyme-mediated color generation using enzymatic catalysis. We also describe future aspects toward advanced colorimetric sensing platforms for toxins based on the unique properties of nanomaterials.

2 Representative Characteristics of Nanomaterials and Bioreceptors for Nanomaterial-Mediated Colorimetric Toxin Detection

Conventionally, the limulus amebocyte lysate (LAL) method was used for colorimetric toxin detection, based on the phenomenon of generating opaque gel clots provoked by the reaction between endotoxins and blood cells of the horseshoe crab (*Limulus polyphemus*). This simple technology was endorsed by the US Food and Drug Administration (FDA), and has broadly been applied as a standard colorimetric method for endotoxin detection. However, the method is considered a qualitative approach that confirms the presence of toxins, but not considered a quantitative technique [11]. To circumvent the shortcomings of the LAL assay, an enzymatic immunoassay (i.e., ELISA) or enzyme-mediated

biosensors were developed and used. Although enzyme-based strategies enable quantitative and sensitive determination, and are amenable for high-throughput assays, the intrinsic instability of enzymes during operation and storage has hindered practical applications [11, 12]. Therefore, there is a great need to develop more advanced colorimetric strategies for toxin detection that are stable even under harsh environments and are feasible for quantitative assays. Recently, diverse nanostructures have been extensively employed for developing colorimetric toxin detection strategies that meet the above requirements. In this section, we describe the representative characteristics of nanostructures, including noble metal nanoparticles and nanozymes as well as bioreceptors, which are used for nanomaterial-mediated colorimetric toxin detection.

2.1 Characteristics of Noble Metal Nanomaterials and Nanozymes to Produce Colorimetric Signal

Noble metal nanoparticles have desirable physicochemical and optoelectronic characteristics that can be deployed as signal transducers [13]. They have been of considerable interest as colorimetric signal-generating materials for POC biosensors in food analysis, including toxin and pathogen detection, depending on their remarkable optical properties owing to their distinct surface plasmon behaviors [9, 14]. In principle, metallic nanoparticles with sizes smaller than the wavelength of light show strong dipolar excitation in the form of localized surface plasmon resonance (LSPR), which is the result of the interaction between the incident electromagnetic wave of light and the surface electron in a conductive band [14, 15]. The spectral properties of LSPR rely heavily on the size, geometry, and composition of nanostructures, and their changes lead to specific surface polarization. In addition, the inter-particle distance as well as the dielectric environment also affect the optical performance of LSPR [14, 15]. Noble metal nanoparticles composed of gold and silver are the most widely employed for LSPR-based sensors owing to their energy levels of d–d transitions that exhibit LSPR in the visible region of the spectrum. In addition, the interaction between incident light and conductive nanoparticles leads to the absorption or scattering of photons [14]. For instance, small and regular gold nanoparticles (AuNPs) are prone to absorb photons, whereas larger and irregular AuNPs tend to scatter them [15]. Silver nanoparticles (AgNPs) possess higher extinction coefficient as well as better sensitivity to the changes of refractive index than those of AuNPs; however, they are not stable over long periods of time under ambient conditions, and thus, require further modifications for the stabilization [16]. Thus, AuNPs have been more extensively utilized for biological applications owing to their easy synthesis, good biocompatibility, inert properties, and facile thiol-mediated interactions,

which are advantageous for inducing binding events with target biomolecules. Monodisperse AuNPs with a diameter of 10–30 nm in solution exhibit a reddish color, which is attributed to the absorption in the blue–green region of the spectrum (~450 nm), while red light (~700 nm) is reflected. The increase in size and aggregation of AuNPs results in the red-shift of the extinction band, resulting in a blue or purple-colored solution [15]. The dispersion-state alteration of AuNPs can change the color of the solution and has been frequently employed for colorimetric signal transduction from the target toxin in a sample.

Natural enzymes can catalyze a variety of biochemical reactions with high substrate specificity under mild conditions, and thus, have been broadly employed in many biotechnological fields [17]. However, their easy denaturation and high production/purification costs hamper practical utilization. As a promising alternative to natural enzymes, nanozymes have attracted much attention owing to their advantageous characteristics, such as high stability even under harsh conditions, tunable catalytic activity comparable to that of natural enzymes, as well as ease of mass production at low costs [12, 17, 18]. Until now, diverse types of nanostructures in the type of metal, metal oxide, metal chalcogenide, carbon, and their hybrids have been extensively reported as nanozymes [17, 19, 20]. Since nanozymes generally catalyze oxidation/reduction reactions, they have been the most extensively applied in the development of biosensors or immunoassays to generate detectable signals by catalyzing the oxidation/reduction reaction of the employed substrate. To produce colorimetric signals, specific chromogenic substrates such as 3,3',5,5'-tetramethylbenzidine (TMB), 2,2'-azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS), *o*-phenylenediamine (OPD), and di-azobenzene (DAB) were employed, yielding blue, green, orange, and brown colors, respectively, from the reaction catalyzed by oxidase- or peroxidase-like nanozymes [21]. Based on these features to generate and amplify colorimetric signals toward target molecules with superior operational stability, diverse nanozymes have been used to develop efficient colorimetric methods for toxin detection, and further research is being conducted in this field [18, 22–26].

2.2 Essential Utilization of Bioreceptors

For specific recognition of target toxins in samples possibly containing many interfering substances, bioreceptor molecules such as antibodies, aptamers, specific polymeric structures, such as molecular imprinted polymers, and several small molecules should be involved in assay development [27]. Among the aforementioned receptors, antibodies and aptamers have frequently been used with nanomaterials for detecting mycotoxins, cyanotoxins, algae, and bacterial toxins [27–30]. Antibodies are conventional biorecognition

units developed by the immune system of animals. They generally show high affinity toward the target antigen; however, their performances are sometimes hindered owing to their intrinsic limitations such as thermal instability, low solubility, and tendency for thermal aggregation [10]. Like antibodies, aptamers show high affinity toward their targets. They are single-stranded oligonucleotides developed in vitro via the systematic evolution of ligands by exponential enrichment (SELEX). Compared to antibodies, aptamers have several merits, including ease of synthesis and modification, high stability, relatively low costs of production, and high binding affinity via successful screening [10]. Depending on the capability of receptors to specifically bind target molecules, appropriate colorimetric toxin detection methods can be established.

3 Recent Examples of Nanomaterial-Mediated Colorimetric Toxin Detection

To date, many different nanostructures have been utilized to establish colorimetric toxin detection. In this section, we briefly summarize recently reported and representative examples of nanomaterial-mediated colorimetric toxin detection. We categorize our discussions into three main groups, including fungal, algal, and bacterial toxins.

3.1 Colorimetric Detection of Fungal Toxins

Fungal toxins, usually known as mycotoxins, are secondary metabolites from fungal species, such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* [3]. These toxins are poisonous to humans and livestock, and commonly contaminate foods such as peanuts, maize, rice, and other cereals during harvesting, transportation, and storage [4]. Aflatoxin (AF), ochratoxin A (OTA), and zearalenone (ZEN) are the most studied toxins owing to their impact on food safety and economic damage. Among the many types of AF, aflatoxin B1 (AFB1) is the most powerful natural carcinogen and the most toxic mycotoxin [10]. Luan et al. [31] established a colorimetric sensor for AFB1 utilizing salt-induced aggregation of AuNPs with DNA aptamers as bioreceptors. DNA aptamers for AFB1 were adsorbed onto the surface of AuNPs via electrostatic interaction, preventing aggregation of the nanoparticles, even in the presence of a high amount of NaCl. However, in the presence of the target AFB1, the conformation of the DNA aptamers changed and eventually detached from the AuNPs, consequently leading to the aggregation of AuNPs. It induces the corresponding color change from red to blue–purple, which can be clearly detected by the naked eye. Based on this strategy, this study showed a satisfactory result with an LOD of 0.025 ng/mL,

which is 20-fold lower than the maximum allowed level of AFB1 in edible foods (0.5 ng/mL) (established by China and European Commission). Wang et al. [32] also developed a sensitive method for AFB1 detection by exploiting a gold enhancement strategy with magnetic bead-mediated sample enrichment. The detection of the assay was based on a competitive immunoassay, in which AFB1-bovine serum albumin (BSA) conjugate-modified magnetic beads were employed as capture probes, while AFB1 antibody-modified AuNPs (Ab-AuNPs) played a dual role as detection probes and in signal transduction. Through the competitive immune reaction of free AFB1 in the sample and immobilized AFB1 on magnetic beads toward the antibody on AuNPs followed by magnetic separation, the recognition event between free AFB1 and detection antibodies on AuNPs was converted into a colorimetric response made from gold enhancement. Based on the proposed strategy, the study showed remarkable sensitivity by yielding an LOD as low as 7 pg/mL via absorbance measurement using spectroscopy. Ji et al. [33] reported the utilization of gold nanoflowers (AuNFs) in lateral flow immunochromatographic test strip (LFA) for AFB1 sensing. AuNFs with multiple branches showed enhanced optical signals due to their higher extinction coefficient compared to that of typical AuNPs. Additionally, AuNFs had a higher surface area than AuNPs, making them more suited to achieve a higher capacity of antibody immobilization. Thus, the employment of AuNFs as a colorimetric signal generator dramatically improved the sensitivity of the sensor, resulting in an LOD as low as 0.32 pg/mL from real samples extracted from rice.

Nanozymes have also been extensively employed for the colorimetric detection of fungal toxins. Lai et al. [22] developed a nanozyme-mediated strategy to visually detect AFB1 (Fig. 1). The method was based on the dissolution of MnO_2 nanoflakes, which originally showed peroxidase-like activity, into Mn^{2+} in the presence of ascorbic acid, leading to the loss of activity. Specifically, free AFB1 in the sample and immobilized AFB1 on BSA-modified magnetic beads (AFB1-MB) were competitive antigens for the AFB1 antibody, which was immobilized on ascorbate oxidase-conjugated AuNPs (Ab-AuNPs-AOx). As the concentration of AFB1 in the sample increased, the amount of conjugate between AFB1-MB and Ab-AuNPs-AOx decreased, resulting in less AOx captured by magnetic separation. With less-captured AOx, the degradation of ascorbic acid was hindered and a relatively higher concentration of ascorbic acid remained, leading to the effective dissolution of MnO_2 nanoflakes, thus inhibiting their peroxidase activity. Consequently, the AFB1 level was quantitatively determined by the concomitant inhibition of the peroxidase activity of the MnO_2 nanoflakes, which could be monitored by the reduced intensity of blue color using TMB as a colorimetric substrate. This nanozyme-mediated strategy was further

successfully applied to detect AFB1 in peanuts with an LOD of 6.5 pg/mL, which is comparable to that obtained from commercial ELISA kits.

Ochratoxin A (OTA), a secondary metabolite originating from fungal species such as *Aspergillus* and *Penicillium*, is considered to be the major metabolite in the Ochratoxin family, and is classified as a possible carcinogen and teratogen [34]. A variety of foods and beverages, such as maize, wheat, peanuts, cocoa, wine, and juice, have been frequently contaminated by OTA [35]. During the processing steps, OTA is not generally denatured, which makes it more dangerous to humans [26]. Recently, various research studies have focused on colorimetric detection of OTA with low LODs [18, 26, 35–37]. Tian et al. [37] recently reported a colorimetric method for OTA detection based on the structural switching of aptamers that led to the metallization of gold nanorods (AuNRs) with the help of alkaline phosphatase (ALP). In this work, aptamer DNA toward OTA was first immobilized on magnetic beads, where ALP-incorporated oligonucleotides were complementary hybridized with the aptamer. In the presence of OTA, the aptamer bound to OTA, which lead to the release of ALP-incorporated oligonucleotides. The liberated ALP then catalyzed the conversion of ascorbic acid 2-phosphate into ascorbic acid, which was able to reduce silver ions (Ag^+) to form a metallic silver shell on the surface of AuNRs, resulting in a blue-shift of the LSPR peak of AuNRs and a concomitant color change. Therefore, as the concentration of OTA increased, the amount of released ALP correspondingly increased, resulting in clear color changes of AuNRs with an LOD of 9 nM, which is advantageous for on-site determination of OTA. In another study, the oxidase-like activity of the spinel MnCo_2O_4 -type nanozyme was utilized to colorimetrically determine OTA (Fig. 2) [18]. This strategy was based on the reversible inhibition of the catalytic activity of the nanozyme by the attachment/detachment of the OTA–aptamer on the MnCo_2O_4 surface via the aptamer–target binding event. The attachment of the aptamer to the nanozyme suppressed its catalytic activity to oxidize TMB, resulting in a lowered colorimetric signal. However, in the presence of target OTA, the high affinity between the aptamer and the target OTA induced the release of the aptamer from the nanozyme surface, which recovered the activity, leading to an increased colorimetric response. Using this strategy, a low LOD of 0.08 ng/mL was achieved while detecting OTA in maize samples, with sufficiently high detection precision and reproducibility, proving the potential to colorimetrically analyze trace amounts of OTA in food samples. Wang et al. [26] also developed a peroxidase-like nanozyme-mediated biosensor for monitoring OTA level with high selectivity. In this work, Fe_3O_4 -doped gold nanoparticles ($\text{Au}@\text{Fe}_3\text{O}_4$ NPs)

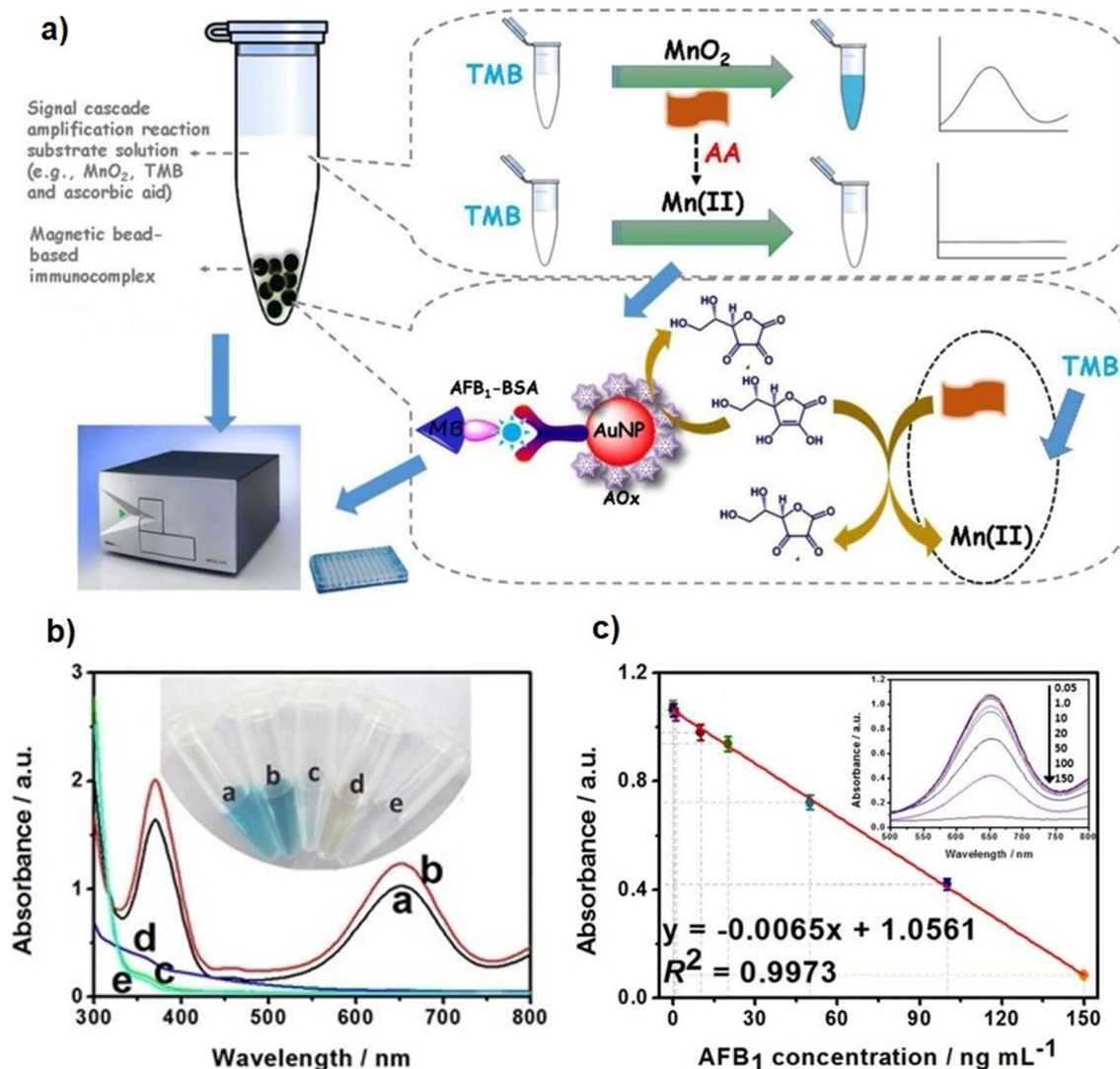


Fig. 1 **a** Schematic illustration representing MnO_2 nanoflake-based colorimetric immunoassay on bovine serum albumin (BSA)-AFB₁-modified magnetic beads by utilizing AOx/AFB₁ antibody-tagged AuNPs with enzyme cascade enhancement strategy and the MnO_2 -TMB system, **b** UV-Vis absorption spectra of (a) AOx + AA + MnO_2 + TMB, (b) AOx + MnO_2 + TMB,

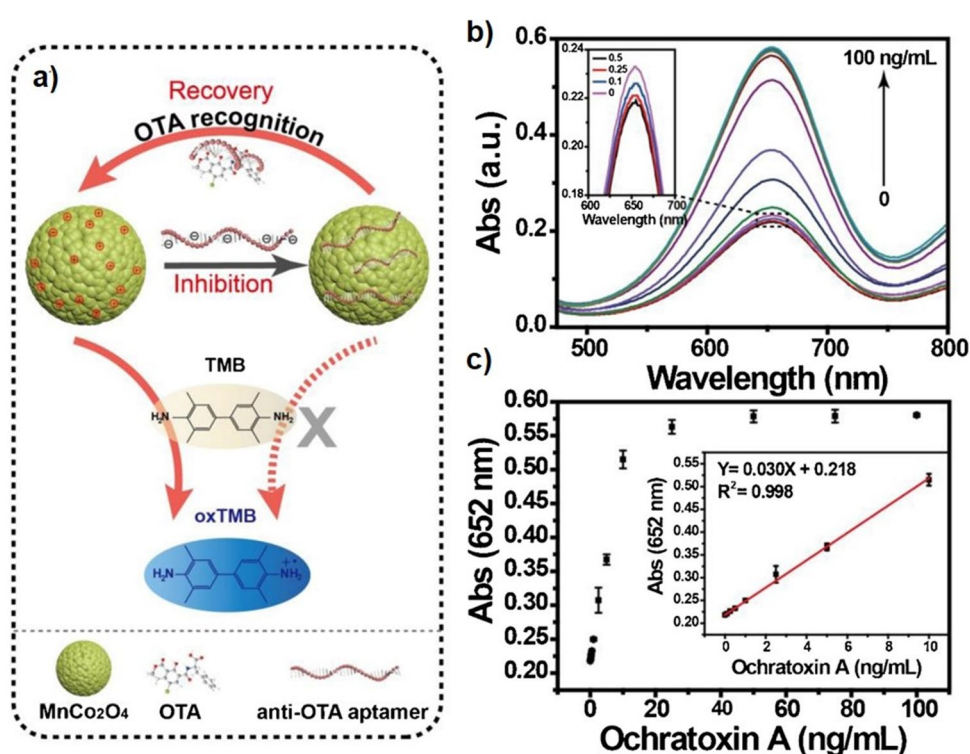
(c) AA + MnO_2 + TMB, (d) AOx + MnO_2 , and (e) AOx + TMB (insets are the corresponding images), and **c** absorption spectra of MnO_2 -TMB-mediated colorimetric assay toward AFB₁ with different concentrations (insets are the corresponding linear curve). AOx ascorbate oxidase, AA ascorbic acid. [22] Reproduced with permission from Elsevier

exhibited peroxidase-like activity and magnetic properties, which could be used for signal transduction and magnetic separation, respectively. In the presence of target OTA, the formation of the OTA–aptamer complex led to the liberation of Au@Fe₃O₄ NPs. Through magnetic enforcement, the collected Au@Fe₃O₄ NPs then catalyzed the oxidation of TMB, resulting in a blue-colored product that could be detected by the naked eye. The method enabled sensitive determination of OTA as low as 30 pg/mL, and was further applied to detect OTA in real samples including peanut and corn with sufficiently high recoveries (92–108%)

and coefficients of variation (< 8.4%), demonstrating its acceptable reliability and precision.

Zearalenone (ZEN) is an estrogenic toxin derived from several *Fusarium* species and primarily produced by *Fusarium graminearum* [4, 24]. Numerous in vivo studies have demonstrated that ZEN is likely to provoke hormonal imbalance owing to its structural similarity to natural estrogens [3]. High levels of ZEN in feed may lead to the disruption of conception in domestic animals including pigs, cows, and other bovine species [4], and thus, the allowed maximum level of ZEN in the feed is restricted

Fig. 2 **a** Schematic illustration representing OTA detection mechanism based on the utilization of oxidase-mimicking MnCo_2O_4 nanozyme, **b** UV–Vis spectra of the absorption corresponding to diverse concentrations of OTA, and **c** calibration curve of the absorbance of oxidized TMB versus OTA concentration. [18] Reproduced with permission from Elsevier



to the range of 20–1000 $\mu\text{g/kg}$ worldwide. Peroxidase-like AuNPs were exploited to colorimetrically detect ZEN based on the inhibition/restoration of the catalytic activity via attachment/detachment of the corresponding aptamer on the surface of the nanozyme [24]. By measuring the recovered colorimetric signal in the presence of ZEN, the ZEN content in corn and corn oil samples was successfully determined with an LOD as low as 10 ng/mL, which is comparable to that obtained using the conventional ELISA methods. Zhang's group also reported AuNP-mediated paper-based lateral flow strips for simultaneously monitoring OTA and ZEN using a competitive immunoassay [38]. Two competitive antigens (OTA-BSA and ZEN-BSA) were immobilized onto a nitrocellulose membrane to form two test lines (T1 and T2 line), while anti-mouse IgG was printed onto the control line (C line). Two kinds of AuNPs-antibody conjugates (AuNPs-OTA antibody and AuNPs-ZEN antibody) were presented in the conjugate pad, which could move by capillary force while capturing their target toxin in sample solutions. Consequently, without any toxin, all the AuNPs-antibody conjugates were trapped by the capturing reagents, and thus, three vivid red lines (T1, T2, and C lines) were generated, whereas a sample containing both toxins gave only one red line, observed on the C line only, because there were no available binding sites on either T line. If two lines appeared (line C and line T), either OTA or ZEN would exist in the sample. This strip sensor enabled sensitive

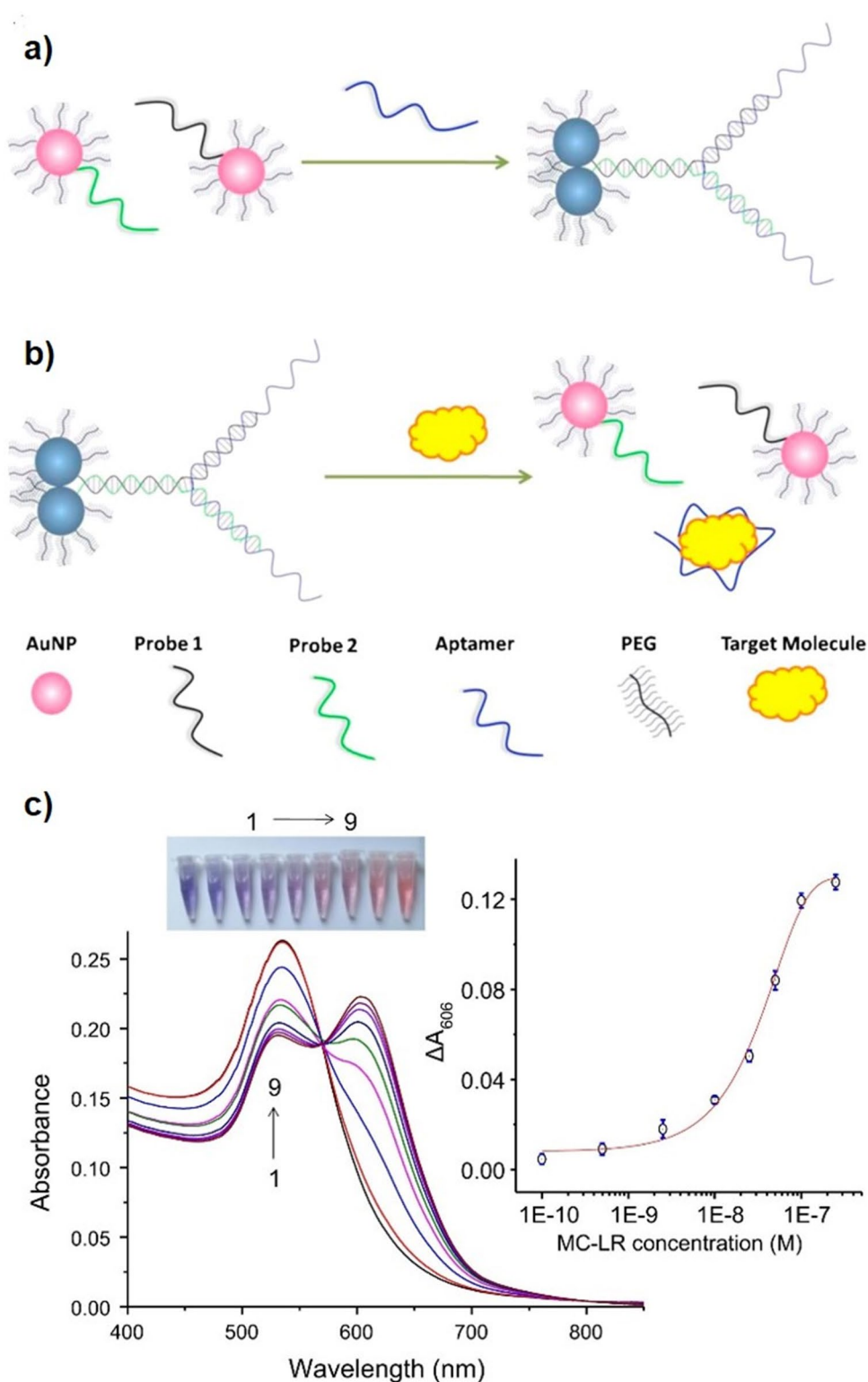
detection of both toxins, yielding LODs for OTA and ZEN to be 0.7697 and 1.2000 $\mu\text{g/kg}$, respectively, confirming its practical utility.

3.2 Colorimetric Detection of Algal Toxins

Algal toxins are organic molecules secreted from different algal species during their blooming process. Algal toxins can be found in aquatic ecosystems, such as fresh, brackish, and marine waters [39]. Harmful algal blooms (HABs) can produce many toxic substances that accumulate in shellfish and marine life, and can reach humans through food webs. These biotoxins are harmless to carriers (e.g., shellfish), but are likely to be lethal or poisonous to humans even at small concentrations. The consumption of seafood-containing algal biotoxins can lead to several syndromes, such as PSP, DSP, and NSP [5].

Microcystins are toxic compounds produced by cyanobacteria, also known as blue-green algae, which inhibit phosphatase activity and induce liver dysfunction and tumors [40]. More than 80 microcystin congeners have been identified, and among them, microcystin-LR (MC-LR), in which L is Leu and R is Arg, is most dominant and poisonous. According to the World Health Organization (WHO), the tolerable thresholds for MC-LR in water and daily intake are 1 $\mu\text{g/L}$ and 0.04 $\mu\text{g/kg}$, respectively [23]. Therefore, it is necessary to develop reliable, sensitive, and portable biosensors that can be conveniently used to

Fig. 3 **a** Formation of AuNP dimers in the presence of aptamers and **b** the disassembly of AuNP dimers in the presence of target toxin, and **c** absorption spectra for the detection of target MC-LR. The top left inset is the photograph of the corresponding reaction solution and the top right inset is the calibration plot of absorbance versus MC-LR concentration. [13] Reproduced with permission from Elsevier



monitor MC-LR frequently. In this regard, Chen's group reported a sensitive method for MC-LR detection within 5 min based on the disaggregation of AuNPs dimers, which induced the shift in LSPR (Fig. 3) [13]. In this work, the AuNP monomer was first asymmetrically modified with

poly (ethylene glycol) and oligonucleotides to prevent the formation of aggregates. Then, the oligonucleotide-modified AuNP monomer formed a dimeric form in the presence of the MC-LR aptamer, which induced a color transition from red to blue. In the presence of MC-LR, the

structure of the aptamer was changed to bind the target MC-LR and induce the dissociation of the AuNP dimer into the monomer. Consequently, the MC-LR level was determined by observing the color transition from blue to red. Uniquely prepared double-integrated nanozymes incorporating both DNAzyme and $\text{Cu}(\text{OH})_2$ nanocages were also utilized in the colorimetric immunoassay to determine MC-LR [23]. To construct a competitive immunoassay, antigenic molecules were first captured on the reaction well, followed by the addition of sample solutions containing target MC-LR as well as the primary antibody. The MC-LR in the sample and captured antigen on the reaction well competitively interact with the primary antibody. As a result, a higher amount of MC-LR in the sample results in less amount of primary antibodies interacting with the captured antigen on the reaction well. Subsequently, secondary antibodies conjugated with $\text{Cu}(\text{OH})_2$ nanocages with DNA primers were incubated to react with the primary antibody present in the reaction well. Finally, amplification of DNA primers was performed by hybridization chain reaction to obtain numerous oligonucleotide products working as DNAzymes with the help of heme. Since both $\text{Cu}(\text{OH})_2$ nanocages and DNAzymes showed excellent peroxidase activity to catalyze the oxidation of ABTS substrate to produce a green color, target MC-LR was successfully determined by the lowering of the green color intensity to as low as 6 ng/mL.

Okadaic acid (OA) is a marine toxin originating from some algal species such as dinoflagellates (e.g., *Dinophysis*) and generally accumulates in shellfish. OA can strongly inhibit the activity of serine/threonine protein phosphatase, which leads to hyperphosphorylation and dysregulation of Na^+ secretion. As a result, humans poisoned by OA generally suffer from diarrhea, one of the symptoms of DSP [28, 41]. For the colorimetric detection of OA, a competitive enzyme-linked aptamer-based assay utilizing the color transition by AuNP aggregation was reported [28]. Aptamers were first immobilized on the reaction well, followed by the addition of complementary oligonucleotide conjugated with catalase and the test sample. If OA is present in the test sample, it competes with the complementary oligonucleotide for binding aptamers on the reaction well. Consequently, a higher amount of OA in the sample results in less amount of oligonucleotide conjugated with the catalase in the reaction well, which hinders the conversion of H_2O_2 into H_2O . A high concentration of H_2O_2 , originating from a high amount of OA, can reduce gold trichloride acid to non-aggregated AuNPs, and the resultant solution is red in color. On the other hand, in the absence of OA, a large amount of catalase could consume H_2O_2 , which induces aggregated AuNPs to yield a blue color. Consequently, this competitive assay yielded an LOD of 0.01 ng/mL with sufficiently high reproducibility and recovery.

Tetrodotoxin (TTX) is a notorious non-protein marine toxin that was first isolated from pufferfish. It is roughly 1200-fold more poisonous to humans than cyanide, and there is no known antidote [42]. For the detection of TTX simultaneously with OA, an AuNP-modified LFA strip was reported for a competitive assay [43]. In this study, AuNPs conjugated with antibody toward either TTA or OA (AuNPs-Ab) were employed to capture their corresponding target toxins. Two T lines were formed, each of which was composed of either a TTX-BSA conjugate or OA-BSA conjugate, while one C line was composed of mouse IgG antibody. In the presence of toxins in samples (TTX and/or OA), AuNPs-Ab specifically bound to their target toxin, and thus, no residual AuNPs-Ab remained for binding TTX-BSA and/or OA-BSA conjugate on the T line. Consequently, a negligible red band appeared in the T line depending on the type of toxin in the sample solution. On the other hand, in the absence of any toxin, there were two red T lines. The red band in the C line always appeared to confirm the right working of the strip assay. This strategy enabled rapid monitoring of both TTX and OA within 10 min with LODs of 15 ng/mL and 0.75 ng/mL, respectively.

Saxitoxin (STX), which is responsible for PSP, is a marine neurotoxin secreted by dinoflagellates and accumulates in many marine organisms, primarily shellfish. Patients poisoned by STX may suffer fatalities arising from cardiovascular shock and respiratory paralysis. Thus, the European Food Safety Authority (EFSA) has suggested that the tolerable threshold of STX in seafood should be set to below 75 $\mu\text{g/kg}$ [44]. In a recent study, gold nanorods (GNRs) were employed as signal transducers with aptamers as biorecognizing elements for STX [29]. In the presence of STX, aptamers, which were originally conjugated on the surface of GNR, were subjected to structural transformation to bind target STX, resulting in a shift in the wavelength of the absorption spectrum of GNR. Using this strategy, STX was determined down to 2.46 $\mu\text{g/L}$, which satisfies the EFSA standard.

3.3 Colorimetric Detection of Bacterial Toxins

Staphylococcal enterotoxins (SEs) produced by *Staphylococcus aureus* are well-known heat-resistant protein-based toxins [25]. Among them, staphylococcal enterotoxin B (SEB) is considered most potent and a major causative agent of foodborne illness. SEB can provoke infection at nanogram levels and resist degradation to hydrolyzing enzymes in the gastrointestinal tract, such as pepsin and trypsin. Furthermore, the virulence of SEB can persist even after heat treatment [25, 45]. The lethal dose of SEB in aerosol exposure is reported to be as low as 0.02 $\mu\text{g/kg}$ and is likely to induce vomiting at an exposure level of 0.4 ng/kg in humans [46]. Hence, it is necessary to develop methods that

are rapid, reliable, sensitive, and affordable in the detection of SEBs. Kingston's group developed a sensitive biosensor to detect SEB utilizing AuNPs as signal transducers [45]. The method was based on the color change of AuNPs in solution due to the aggregation/disaggregation mechanism. Aptamers specific for SEB were selected and conjugated with AuNPs to prevent aggregation of nanoparticles. In the presence of target SEB, the structure of the aptamers was distorted, and, thus, led to the aggregation of AuNPs to induce color change from red to blue. With this approach, SEB was detected at concentrations as low as 50 ng/mL and 0.5 ng/mL, with the naked eye and spectrophotometer, respectively. SEB was also visually detected with a much lower LOD of 0.12 ng/mL (Fig. 4) [25]. In this work, the eggshell membrane-embedded gold nanoclusters (Au-ESM) having glutathione-mediated peroxidase-like activity were utilized to decompose H_2O_2 , which had a crucial role in the growth of AuNPs. In the absence of target SEB, the unoccupied aptamer-modified Au-ESM showed catalytic activity to decompose H_2O_2 , which induced the growth of AuNPs. Conversely, in the presence of SEB, it specifically interacted with aptamer-modified Au-ESM, leading to the

formation of SEB-aptamer-Au-ESM, which hampered the peroxidase activity of Au-ESM. Consequently, it could be seen that the color gradually changed from violet to red with increasing concentration of SEB. The detectable threshold of this method was determined to be 0.12 ng/mL, and was successfully applied to detect SEB in samples such as flour, maize, and rice.

Staphylococcal enterotoxin A (SEA) has also been broadly examined due to its causative capacity in toxic shock syndrome as well as food poisoning [47]. Boujday's group reported an effective LSPR-based detection method for SEA using core-shell nanoparticles composed of gold and silver [48]. In this study, two different types of core-shell structure, including Au@AgNPs in which a gold core was covered by a silver layer and Ag@AuNPs where silver served as a core with a gold layer, were prepared and utilized to obtain optimized LSPR-based optical response toward the target SEA. By controlling the thickness of the shells and surface functionality to efficiently conjugate SEA antibody, the LSPR band of the core-shell nanoparticles was precisely controlled. Upon the addition of SEA, large red shifts of the LSPR band were observed

Fig. 4 **a** Schematic illustration for the preparation of peroxidase-mimicking Au-ESM and their application to colorimetrically detect SEB, **b** calibration plot for the colorimetric detection of SEBs with corresponding real images, and **c** real images for the detection of SEBs spiked in food samples. [25] Reproduced with permission from Elsevier

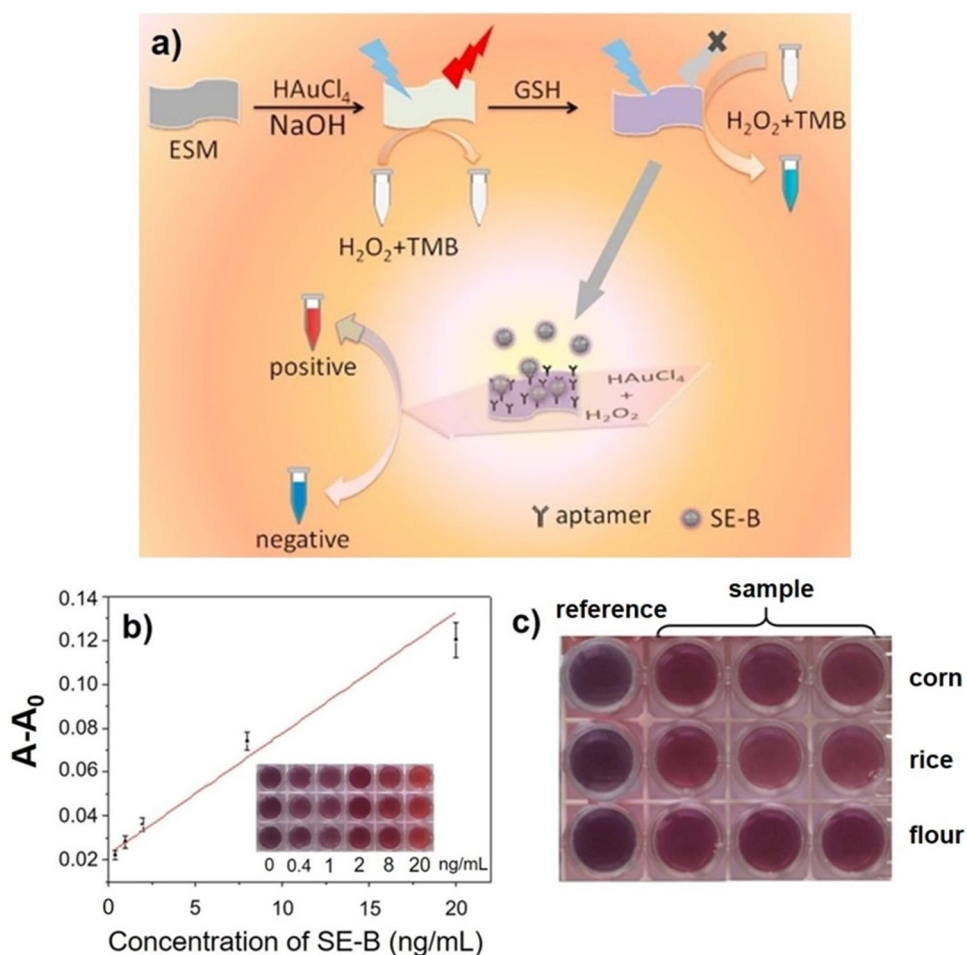


Table 1 Diverse studies on nanomaterial-mediated colorimetric detection of toxins

Strategy	Target toxin	Nanostructure	Bioreceptor	LOD	Real sample	References
Catalysis	AFB1	MnO ₂	Antibody	6.5 pg/mL	Peanut	[22]
LSPR	AFB1	AuNPs	Aptamer	0.025 ng/mL	Rice	[31]
LSPR	AFB1	AuNPs + MBs	Antibody	7 pg/mL	Maize	[32]
LSPR	AFB1	AuNFs	Antibody	0.32 pg/mL	Rice	[33]
Catalysis	OTA	MnCo ₂ O ₄	Aptamer	0.08 ng/mL	Maize	[18]
Catalysis	OTA	Au@Fe ₃ O ₄	Aptamer	30 pg/mL	Peanut, corn	[26]
LSPR	OTA	AuNRs	Aptamer	9 nM	Grape juice	[37]
LSPR	OTA	AuNPs	Antibody	0.7697 µg/kg	Corn	[38]
Catalysis	ZEN	AuNPs	Aptamer	10 ng/mL	Corn, corn oil	[24]
LSPR	ZEN	AuNPs	Antibody	1.2 µg/kg	Corn	[38]
LSPR	MC-LR	AuNP dimers	Aptamer	0.05 nM	Water samples collected from local rivers, lakes, and ponds	[13]
Catalysis	MC-LR	Cu(OH) ₂ NC@DNAzyme	Antibody	6 ng/mL	Tap water and the water from the West Lake	[23]
LSPR	OA	AuNPs	Aptamer	0.01 ng/mL	Clams	[28]
LSPR	OA	AuNPs	Antibody	0.75 ng/mL	Clams, green mussels, razor clams	[42]
LSPR	TTX	AuNPs	Antibody	15 ng/mL	Clams, green mussels, razor clams	[42]
LSPR	STX	AuNRs	Aptamer	2.46 µg/mL	Mussel	[29]
Catalysis	SEB	Au-ESM	Aptamer	0.12 ng/mL	Rice, corn, and flour	[25]
LSPR	SEB	AuNPs	Aptamer	0.5 ng/mL	Milk	[45]
LSPR	SEA	Au@AgNPs	Antibody	0.2 nM	–	[48]
LSPR	SEA	Ag@AuNPs	Antibody	0.4 nM	–	[48]
LSPR	Enterotoxin from <i>E. coli</i> O78:H11	Au@Lac-CEW NPs	Lac-CEW	31 nM	Apple juice	[49]

AFB1 aflatoxin B1, *OTA* ochratoxin A, *ZEN* zearalenone, *MC-LR* microcystin-LR, *OA* okadaic acid, *TTX* tetrodotoxin, *STX* saxitoxin, *SEB* staphylococcal enterotoxin B, *SEA* staphylococcal enterotoxin A, *AuNPs* gold nanoparticles, *MBs* magnetic beads, *AuNRs* gold nanorods, *Au-ESM* eggshell membrane-template gold nanoclusters; Au@AgNPs: Au–Ag core–shell nanoparticles, Ag@AuNPs Ag–Au core–shell nanoparticles, *Lac-CEW* lactose-functionalized chicken egg white protein, *Au@Lac-CEW NPs* Lac-CEW-functionalized gold nanoparticles

spectroscopically and the LOD was determined to be 0.2 nM and 0.4 nM, with Au@AgNPs and Ag@AuNPs, respectively. Between the two core–shell nanoparticles, the Ag@AuNPs displayed a more distinct color change from orange to red, which can be distinguished with naked eyes. Another diarrhea-causing enterotoxin secreted from pathogenic *E. coli* O78:H11 was also visually detected using a glycosylated protein-linked AuNPs [49]. Instead of conventional bioreceptors like antibodies, lactose-functionalized chicken egg white protein (Lac-CEW) was conjugated on AuNPs (Au@Lac-CEW NPs) to specifically detect the target enterotoxin. In the absence of enterotoxin, the color of the reaction solution changed from red to purple because of the self-aggregation of Au@Lac-CEW NPs under acidic pH conditions. In contrast, the color of the solution remained red in the presence of target enterotoxin, since the complex consisting of Au@Lac-CEW NPs and enterotoxin could withstand low pH conditions and effectively prevent their aggregation. Based on the phenomena, the strategy enabled sensitive detection of target enterotoxin as low as 31 nM.

4 Conclusions and Prospects

Food toxins are an enduring concern for human health worldwide. These toxins are not easily destroyed by conventional food processing, including heat treatment, and most non-protein toxins seem to be invisible to the human immune system. Some protein toxins, including SEBs, can bypass the effect of the hydrolyzing enzyme in the gastrointestinal tract and provoke disease. Considering their toxicity, which could lead to fatalities, and their ability to induce serious diseases at very small doses, there is a pressing need to develop reliable, sensitive, and convenient detection methods that can be used for routinely checking the presence of toxins in foods. In this regard, the use of nanomaterials and appropriate biorecognition elements to develop a colorimetric assay system significantly enhances the detection performance for toxins (Table 1).

For extensive practical applications of nanomaterial-mediated colorimetric toxin detection strategies, we believe that the following technical issues should be urgently addressed: (1) Until now, relatively limited types

of nanostructures, including noble metal nanoparticles or nanozymes, have been utilized to develop colorimetric toxin detection strategies. Therefore, further advancement in the development of novel nanostructures with higher sensitivity and specificity by generating a much clearer colorimetric response would be highly desirable. (2) For practical usage to detect toxins on-site, sufficiently high reliability and reproducibility of the nanomaterial-mediated colorimetric method should be obtained. To achieve this goal, it is necessary to develop a fully demonstrated protocol for synthesizing mono-sized nanostructures with their appropriate conjugating method with bioreceptors. (3) In addition, it is important to develop a robust assay method to simultaneously detect multiple toxins, since many foods can be contaminated by multiple toxins [37]. To this end, novel integration of multiple detection principles in a single assay platform is required. With the above-mentioned studies, we expect that the utilization of nanostructures for the development of colorimetric toxin detection will increase in the near future.

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