Research article

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Black phosphorus-Au nanocomposite-based fluorescence immunochromatographic sensor for high-sensitive detection of zearalenone in cereals

<https://doi.org/10.1515/nanoph-2019-0434>

Received October 18, 2019; revised December 7, 2019; accepted December 13, 2019

Abstract: Rapid and high-sensitive detection of mycotoxins is believed to be of vital importance in assuring food safety. In this study, we developed a novel fluorescence immunochromatographic sensor (ICS) for the mycotoxin of zearalenone (ZEN) in cereals. This was done by using a black phosphorus-Au nanocomposite (BP-Au) as the 2D quenching platform. Herein, gold nanoparticles (AuNPs) were directly reduced on the surface of BP nanosheets (BPNSs) to form BP-Au nanocomposites, showing higher fluorescence (quantum dots, $\lambda_{\text{Em}} = 525 \text{ nm}$) quenching efficiency compared to the BPNSs and AuNPs. The fluorescence quenching efficiency of the prepared BP-Au nanocomposite reached 73.8%, which was 1.73-fold and 1.44-fold higher than AuNPs and BPNSs, respectively. The density functional theory was also successfully used to explore the formation mechanism of the BP-Au nanocomposite. By introducing the quantum dots/BP-Au signal/quencher pair, a high-sensitive fluorescence quenching ICS (B-FICS) was developed for the detection and discrimination of ZEN with the limit of detection of $0.1 \mu\text{g/l}$ in pure working buffer. This was 2.5-fold more sensitive

than AuNPs-based FICS (A-FICS). The B-FICS was successfully applied in real cereals detection with the sample limit of detection of $2 \mu\text{g/kg}$. The successful construction of B-FICS offers a novel method for a rapid and high-sensitive detection of ZEN in cereals. It also provides a new practical application of 2D BPNSs in food safety sensing.

Keywords: black phosphorus; Au nanoparticle; immunochromatographic sensor; zearalenone; food safety sensing.

1 Introduction

Black phosphorus nanosheets (BPNSs) constitute an elementary crystal which was successfully stripped in 2014 [1]. As a new member of 2D nanomaterials, BPNSs can be prepared with high purity [2], and a few-layered BPNS has a natural direct bandgap and high carrier mobility (up to $1000 \text{ cm}^2/\text{Vs}$) with a thickness-dependent bandgap width from 0.3 eV to 2.0 eV [3, 4], allowing broadband absorption from the visible to mid-infrared range. Consequently, the 2D BPNSs demonstrate great potential in field-effect transistors [5], nanoelectronics [6], optoelectronics [7], catalysis [8], energy, etc. Furthermore, BPNSs have emerged as ideal photothermal, photodynamic therapeutic reagents and a photoacoustic imaging material because of their good biocompatibility, high photothermal conversion rate and unique electronic structure [9–12]. Their chemical instability also enables them to serve as a reducing agent to reduce metal nanoparticles on the surface of BPNSs to construct biosensors [13].

The photothermal properties and chemical instability of BPNSs had been used in our previous study to reduce gold nanoparticles (AuNPs) on the surface of BPNSs, and then the prepared BP-Au nanocomposite was successfully applied as a photothermal sensitive probe to develop an innovative photothermal immunochromatographic sensor (ICS) for sensitive detection of

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enrofloxacin residues in animal-derived food samples [14]. It was proved that BPNSs could be used as sensing component in the ICS device. Interestingly, we found that the BP-Au nanocomposite had much higher absorption capacity than AuNPs [15] and BPNSs [16] after introducing AuNPs to BPNSs. It indicated that the BP-Au nanocomposites had strong fluorescence quenching efficiency at certain wavelengths.

Zearalenone (ZEN) is a kind of mycotoxin which is mainly produced by *Fusarium gramineous*. It is widely distributed in cereals, for example corn, wheat, sorghum, and rice. As an exogenous estrogen-like mycotoxin, ZEN can cause high estrogen syndrome in mammals and cause infertility, miscarriage, and stillbirth. It also has genotoxicity, immunotoxicity, cytotoxicity, teratogenic toxicity, etc. ZEN pollution of crops not only greatly undermines agriculture, it also introduces serious and long-term food safety problems and threatens human health. For this reason, it is especially important to ensure that cereals are not contaminated with ZEN. The ICS [17] has received much attention in ZEN sensing due to its advantages of simple operation, rapid detection and low cost [18, 19], and has become one of the most suitable on-site visual inspection tools for ZEN [20, 21].

In this work, AuNPs were directly reduced on the surface of BPNSs to form BP-Au nanocomposites, and the BP-Au nanocomposites demonstrated strong fluorescence quenching efficiency at the wavelength of 515–521 nm. Therefore, the BP-Au nanocomposites were used as a fluorescence quencher of quantum dots (QDs, $\lambda_{\text{em}} = 525$ nm) to develop a novel BP-Au nanocomposite-based fluorescent quenching ICS (B-FICS) for ZEN sensing. As a comparison, AuNPs-based ICS (A-FICS) was also established using AuNPs as the quencher. The developed B-FICS was successfully applied in real cereal samples and validated using the commercial ELISA kit.

2 Materials and methods

2.1 Materials

Gold acid chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), sodium citrate, bovine serum albumin (BSA), and ovalbumin (OVA) N-hydroxysuccinimide, 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide, goat anti-mouse IgG, ZEN, α -zearalenol, α -zearalanol, β -zearalenol, zearalanone, β -zearalanol, and aflatoxin B₁ (AFB₁) were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). BPNSs were purchased from Nanjing XFNANO Materials

Tech Co., Ltd. (Nanjing, China). Carboxyl-functionalized water-soluble ZnCdSe/ZnS QDs were purchased from Wuhan Jiayuan Quantum Dots Co., Ltd. (Wuhan, China). T-2 toxin, ochratoxin A (OTA), deoxynivalenol (DON), and fumonisin B₁ (FB₁) were purchased from Toronto Research Chemicals (Toronto, Canada). Pyridine and carboxymethoxylamine were purchased from TCI Development Co. Ltd. (Shanghai, China). The commercial ELISA test kit was purchased from Pribolab Pte. Ltd. (Singapore). Anti-ZEN monoclonal antibody (Ab) was provided by our laboratory. Nitrocellulose (NC) membranes (HF135 with a capillary flow of 135 s/4 cm) were purchased from Millipore Corporation (Bedford, MA, USA). Sample pad, conjugate pad, absorbent pad and polyvinyl chloride sheets were purchased from Kinbio Tech Co. (Shanghai, China). The FICSs were observed using a portable UV lamp (Shanghai JiaPeng Technology Co., China). The absorbance spectrum was measured with Thermo Scientific Varioskan LUX microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.2 Preparation of the BP-Au nanocomposite-antibody conjugate and AuNPs-antibody conjugate

BP-Au nanocomposites and AuNPs were synthesized according to previous reports with slight modifications [13, 22], and the principles of BP-Au nanocomposite synthesis are shown in Figure 1A. All synthetic details are documented in the electronic Supplementary Material. To explore the formation mechanism of the BP-Au nanocomposite, the first-principles calculations in the framework of the density functional theory (DFT), including atomic structure, total energy, and electron density difference were carried out based on the Cambridge Sequential Total Energy Package (CASTEP).

BP-Au nanocomposite-Ab conjugates (BP-AU-Ab) and AuNPs-Ab conjugates (AuNPs-Ab) were prepared through the physical adsorption method. Specifically, 0–15 μl of 0.2 mol/l K_2CO_3 and 1–10 μl of 1 mg/ml ZEN-Ab were added to 1 ml of BP-Au nanocomposite or AuNPs solution, and the mixture was then incubated for 1 h at room temperature. Next, 10 μl of a 20% BSA aqueous solution and 5 μl of 10% PEG-20000 were added to the above mixture and incubated for 30 min at room temperature. The products were then centrifuged at 20,817 rcf for 15 min at 4°C. The supernatant was discarded, and the precipitate was re-dissolved in 200 μl of working buffer for usage.

Figure 1: Schematic of the developed black phosphorus (BP)-Au nanocomposite-based fluorescent quenching immunochromatographic sensor (B-FICS).

(A) Preparation route of the BP-Au-Ab quenching probe. (B) Construction of the B-FICSs. C-line: control line (quantum dots [QDs]-ovalbumin [OVA]). T-line: test line [a mixture of zearalenone (ZEN)-OVA and OVA-QD].

2.3 Preparation of the fluorescence signal probe (QDs-OVA)

The fluorescence probe was synthesized according to previous reports with slight modification [22], and all synthetic details are reported in the electronic Supplementary Material.

2.4 Preparation of the coating antigen

The coating antigen (ZEN-OVA) was synthesized according to the method described in a previous report [23] with slight modification. Details on this are given in the Supplementary Material.

2.5 Detection procedure

The detection principle of B-FICS is shown in Figure 1B, and the FICS construction details are listed in the Supplementary Material. Firstly, 150 μl of a standard or sample solution and 5 μl of the quenching probe were mixed and dropped onto the sample pad. The results of FICSs were observed after 20 min under a portable UV lamp, and the limit of detection (LOD) was defined as the concentration of ZEN when fluorescence almost disappeared at the T-line.

2.6 Real cereal sample analysis

Referring to the cereal samples, 2.0 g cereal samples were ground and sieved through an 80 mesh sieve; 0.2 g of NaCl were added to a 15 ml centrifuge tube, 4 ml of a 90% acetonitrile-water solution (w/w) was added, and the tubes were vigorously shaken for 5 min. The mixture was then left to stand for a while and the supernatant was diluted for FICS analysis.

3 Results and discussion

3.1 Characterization of the BP-Au nanocomposites

The prepared BPNSs and BP-Au nanocomposites were identified by a transmission electron microscope. The transmission electron microscopy results showed that the AuNPs which were reduced on the surface of BPNSs (Figure 2A) had an average particle size of about 13 nm (Figure 2B and C). The AuNPs were uniformly dispersed on the BPNSs, forming the BP-Au nanocomposite. In addition, the visible-near-infrared absorption spectroscopy (Figure 2D) revealed that the prepared BP-Au nanocomposite had an obvious absorption peak around 518 nm compared with BPNSs, and the absorbance intensity of the BP-Au

Figure 2: Characterization of the prepared black phosphorus nanosheets (BPNSs) and BP-Au nanocomposite.

(A) The transmission electron microscopy (TEM) image of BPNSs. (B) TEM image of the BP-Au nanocomposite. (C) Particle size statistics of Au nanoparticles on the surface of the BP-Au nanocomposite. (D) Visible-near-infrared (Vis-NIR) spectra of BPNS and the BP-Au nanocomposite with the concentration ranging from 12.5 µg/ml to 50 µg/ml.

nanocomposite in the visible-near-infrared region was also significantly enhanced. This phenomenon further illustrated the successful preparation of the BP-Au nanocomposites. The chemical instability of BPNSs allowed the AuNPs to be directly reduced on the surface of the BPNSs at room temperature without any other reducing agents, so that this synthesis method was operable. The linear relationship between the absorbance intensities of BPNSs or the BP-Au nanocomposite and their concentrations, and the spectra overlap of the BP-Au nanocomposite and QDs were analyzed to verify if the prepared BP-Au nanocomposite can be used as a fluorescence quenching probe. The absorbance values of BPNS and the BP-Au nanocomposite at 525 nm and 808 nm increased linearly according to the increase of their concentrations (Figure 3A). The absorbance intensity of the BP-Au was much stronger and faster growing than BPNS at each concentration point, indicating that the BP-Au nanocomposite has more potential to

be applied as a quenching platform in the ICS than BPNSs. The absorption spectrum of the BP-Au nanocomposite and the fluorescence spectrum of the fluorescent donor probe (QDs) are shown in Figure 3B. Both absorption spectra of the AuNPs and the BP-Au nanocomposite can overlap well with the fluorescence spectrum of QDs, but the BP-Au nanocomposite had double the amount of light absorption intensity than the AuNPs at 525 nm (Figure 3B). This indicated that the prepared BP-Au nanocomposite could be used as the quenching probe in a fluorescence resonance energy transfer sensing platform.

3.2 Mechanism of BP-Au nanocomposite formation

The above results confirmed that BPNSs could reduce Au^{3+} to the gold atom, forming a gold nanoparticle structure.

Figure 3: Feasibility analysis of the black phosphorus (BP)-Au nanocomposite quenching probe.

(A) A linear relationship between absorbance intensity (at 525 nm and 808 nm) and BPNS or BP-Au nanocomposite concentrations. (B) Visible-near-infrared (Vis-NIR) spectra of the BP-Au nanocomposite and gold nanoparticles (AuNPs), and the fluorescence spectra of quantum dots (QDs). (The left Y axis is for Vis-NIR spectra and the right Y axis is for fluorescence the emission spectrum of QDs)

In order to explore the formation mechanism, the interactions between BPNSs and Au^{3+} or Au were calculated by the DFT method. This was done to explain the differences of structure, adsorption energy, and electron density.

The first-principles calculations in the framework of DFT, including atomic structure, total energy, and electron density difference, were carried out based CASTEP [24]. The exchange-correlation functional under the generalized gradient approximation [25] with ultrasoft pseudopotentials [26] and the Perdew-Burke-Ernzerhof [27] functional were implemented so that the electron-electron interaction could be described. The energy cut-off for the plane-wave basis set of 320 eV was considered and a k-point set at $1 \times 4 \times 2$ was tested for convergence. A maximum force tolerance of 0.03 eV/Å, an energy tolerance of 1.0×10^{-5} eV/atom and a maximum displacement tolerance of 1.0×10^{-3} Å were set in this study. The vacuum space along with the z direction was set to be 15 Å. The Au atom/ion was placed on the top of the BP. The atoms at the bottom were fixed, while the atoms at the top and the Au atom/ion were relaxed. The adsorption energy (ΔE) of the Au atom/ion on the surface of the BP was defined as:

$$\Delta E = E_{\text{Au}}^* - (E^* + E_{\text{Au}}) \quad (1)$$

where E_{Au}^* and E^* represent the adsorption of the Au atom/ion on BP and the bare BP, respectively. E_{Au} represents the energy of Au atom/ions.

As shown in Figure 4A, the coordination number of each phosphorus atom is 3, and the phosphorus atoms are covalently bonded to form a pleated 2D honeycomb structure. The optimized lattice constant of a single layer of BPNSs is $a = 3.033$ Å and $b = 4.229$ Å. It is similar to previous literature [28]. The optimized structure is shown in Figure 4B and the distances between BPNSs and gold ions

and atoms are 1.497 Å and 1.613 Å, respectively, indicating that BPNSs have attractive effects on gold ions and atoms, but interact better with gold ions. Figure 4C shows the electron density difference analysis; Au ions are more positively charged and have a strong effect on BPNSs in electron-deficient sites. Figure 4D also proves that BPNSs are more attractive to gold ions. Therefore, it is speculated by DFT calculation that after Au^{3+} was reduced by BPNSs, the positive charge of Au atoms and the binding force to the BPNSs declined, so that the Au atoms were aggregated with each other to form AuNPs.

3.3 Evaluation of the quenching ability of the BP-Au nanocomposite

The quenching abilities of AuNPs, BPNSs, and the BP-Au nanocomposite to the QDs ($\lambda_{\text{Em}} = 525$ nm) were analyzed, and the quenching rate was calculated using the following formula:

$$\text{Quenching rate } (R\%) = (I_0 - I_d) / I_0 \times 100\% \quad (2)$$

where I_0 is the fluorescence intensity of QDs (2.5 nmol/l), which are measured at 525 nm without BPNSs, AuNPs or the BP-Au nanocomposite. I_d is the fluorescence intensity of QDs at the same conditions, and it is measured after adding BPNSs, AuNPs or the BP-Au nanocomposite.

The results are shown in Figure 5. The quenching rates of the AuNPs, BPNSs and BP-Au nanocomposite increased linearly with the increase of their concentrations, which was a necessary condition for a nanomaterial to serve as a quenching probe. Conversely, the quenching rate of the BP-Au nanocomposite could reach 73.8%, which was 1.73-fold and 1.44-fold higher than AuNPs ($R\% = 42.6\%$)

Figure 4: The optimized structures of the bare black phosphorus (BP).

(A) (1) top and (2) side views. (B) The optimized structures of the Au atom/ion on BP, where the (1) red and (2) yellow areas represent Au ions and Au atoms, respectively. (C) The electron density difference slices of the BP-Au nanocomposite, where the red and blue areas represent the accumulation and depletion of electrons. (D) Adsorption energy (ΔE) of Au atoms/ions on the surface of BP.

Figure 5: Comparison of the quenching ability of different concentrations of gold nanoparticles (AuNPs), black phosphorus nanosheets (BPNSs), and the BP-Au nanocomposite.

and BPNSs ($R\% = 51.2\%$), respectively. The combination of BPNSs and AuNPs greatly enhanced the fluorescence quenching ability of the quenching probe.

In addition, the chromatographic effects of the quenching probe on the NC membrane were explored. BPNSs did not leave a distinct color at the C- or T-line on the NC membrane under the same conditions after

coupling with Ab and spraying on the NC membrane (Figure S1). This indicated that the pure BPNSs were not suitable for constructing fluorescent quenching probes due to their own dark brown color and the low antibody coupling ability. For the conventional AuNPs-Ab quenching probe, the low concentration of AuNPs (prepared by $4.93 \mu\text{g/ml}$ of HAuCl_4) showed insufficient colorimetric brightness and poor quenching efficiency. In contrast, the BP-Au-Ab probe, which was prepared by the same concentration of HAuCl_4 , showed higher fluorescence absorption ability and a more pronounced colorimetric brightness and quenching efficiency. This indicated that the BP-Au nanocomposites were more suitable for use as the fluorescence quenching probes in the FICS device.

3.4 Sensitivities of the two developed FICS methods

The BP-Au nanocomposite and AuNPs were used as quenching probes based on the above results to establish a novel BP-Au nanocomposite-based FICS (B-FICS) and a traditional AuNPs-based FICS (A-FICS) for the detection of ZEN in a pure working buffer. To obtain the best sensitivity, several experimental conditions were optimized, such as the amount of Ab, the pH condition, the amount of QDs,

and the amount of coated antigen. Detailed optimization conditions are listed in Table S1.

Under the optimal conditions, the B-FICS and the A-FICS were established. For the B-FICS, the BP-Au nanocomposites (prepared by $4.93 \mu\text{g/ml}$ of HAuCl_4) were conjugated with Ab as the fluorescence quenching probe. When observing the BP-Au nanocomposite-based B-FICS under UV light, green fluorescence appeared in the T-line region at $0.1 \mu\text{g/l}$ of ZEN (Figure 6A), so the LOD of the developed B-FICS is defined as $0.1 \mu\text{g/l}$. However, when observing under natural light, the brown band of the T-line still remained when the ZEN concentration was increased to $0.25 \mu\text{g/l}$ and gradually disappeared until the concentration of ZEN was increased to $1 \mu\text{g/l}$, so the LOD of B-FICS under natural light was $1 \mu\text{g/l}$ (Figure 6B). For AuNPs-based A-FICS, a higher concentration of AuNPs (prepared by $9.85 \mu\text{g/ml}$ of HAuCl_4) was used to obtain enough quenching efficiency at the T-line region. Compared with the B-FICS, the A-FICS needs a higher concentration of Ab to form the recognition probe, and also needs a higher concentration of coating antigen to form the T-line capture probe (Table S1). The green fluorescence of the T-line region virtually disappeared when the concentration of ZEN was $0.25 \mu\text{g/l}$ (Figure S2). Therefore, the LOD of the A-FICS was $0.25 \mu\text{g/l}$, which was higher than the LOD of the B-FICS. A fluorescence resonance energy transfer-based B-FICS is much more sensitive for ZEN sensing. This is because the sheet-like

structure of BP-Au nanocomposites provides a stronger antibody loading capacity and quenching ability to form the recognition probes. Therefore, the BP-Au can be used as an ideal quenching platform to develop a sensitive FICS device in food safety sensing.

As can be seen in Table 1, many different kinds of analysis methods have been developed for ZEN detection in previous studies. Among these methods, immunoassays show an obvious advantage in sensitivity compared with other recognition molecule-based methods such as the aptamers. Chromatographic immunoassays have become the ideal on-site detection tools due to the advantages of being easy to operate, portable, fast and of low cost. In addition, the FICS has an incomparable advantage because the fluorescence intensity of the T-line is sensitively correlated with the concentration of the analyte, which can improve the detection sensitivity distinctly. In this study, the BP-Au demonstrates great potential in developing the FICS for ZEN sensing because of its good quenching ability and antibody loading capacity. This developed semi-quantitative B-FICS shows a higher sensitivity than the traditional A-FICS. Although this developed B-FICS is not the most sensitive one compared with some other highly sensitive methods, it is sensitive enough to fulfill the detection requirement according to the maximum residue limits which were set by many regions and countries [36]. In addition, the B-FICS meets the demands of convenience, speediness, economy, and

Figure 6: Detection of zearalenone (ZEN) using Au nanocomposite-based fluorescent quenching immunochromatographic sensors (B-FICSs).

A series of concentrations of ZEN ($0 \mu\text{g/l}$, $0.1 \mu\text{g/l}$, $0.25 \mu\text{g/l}$, $0.5 \mu\text{g/l}$, and $1 \mu\text{g/l}$) was prepared in pH 7.4 PBS (0.01 mol/l) and detected under (A) UV lamp and (B) natural light. A series of concentrations of ZEN was spiked into cereals and used in B-FICS ($0 \mu\text{g/kg}$, $2 \mu\text{g/kg}$, $5 \mu\text{g/kg}$, $10 \mu\text{g/kg}$ and $20 \mu\text{g/kg}$). (C) Maize. (D) Wheat.

Table 1: An overview on recently reported immunoassays for the determination of zearalenone (ZEN).

Proposed assays	Signal materials	Recognition molecules	LOD ($\mu\text{g/l}$)	Ref.
ELISA	Horseradish peroxidase (HRP)	Antibody	2	[29]
	Horseradish peroxidase (HRP)	scFv antibody	2	[30]
Centrifugal platform immunoassay	Cy3	Antibody	0.55	[31]
Electrochemical immunosensor	Screen-printed electrodes/ alkaline phosphatase (ALP)	Antibody	0.25	[32]
Magnetic separation immunoassay	Magnetic bead/ HRP	Antibody	0.098	[33]
Chromatographic assay	Quantum dot nanobeads	Antibody	0.059	[34]
	AuNPs	Aptamer	20	[35]
	QDs	Antibody	1 (visual)	[7]
	AuNPs/QDs	Antibody	0.25 (visual)	This work
	BP-Au/QDs	Antibody	0.1 (visual)	This work

ALP; AuNPs, gold nanoparticles; BP, black phosphorus; LOD, limit of detection; QDs, quantum dots.

efficiency for on-spot detection of ZEN, so the successful construction of B-FICS offers a novel method for rapid and sensitive detection of ZEN in cereals.

3.5 Specificity of the developed B-FICS

Ten different mycotoxins, including five structural analogs of ZEN, were selected to evaluate the specificity of the proposed B-FICS. The results are shown in Figure S3. For the five structural analogues (α -zearalenol, α -zearalanol, β -zearalenol, zearalanone, and β -zearalanol), the fluorescence intensity of the T-line region of the B-FICS appeared when concentrations of the analytes were between 1 $\mu\text{g/l}$ and 2.5 $\mu\text{g/l}$. For AFB₁, T-2 toxin, OTA, DON, and FB₁, no fluorescence signal was observed on the T-line region even when the concentrations of analytes reached 10 $\mu\text{g/l}$. This indicated that the developed B-FICS had little or even no cross-reactions with AFB₁, T-2 toxin, OTA, DON, and FB₁.

3.6 Application of the B-FICS in real sample analysis

Negative maize and wheat samples without ZEN were used for the recovery experiment. After spiking ZEN, an extraction step was done on samples by using the extracted solution and then diluting the extract with PBS buffer. For the negative sample extract, the fluorescence intensity of the T-line region gradually decreased until it disappeared when the sample extract's dilution times increased. This is because the effect of the sample matrix is gradually weakened. When the sample extract was diluted 20-fold, the fluorescence signal of the T-line was completely quenched. Under the optimized conditions, the

fluorescence of the T-line region appeared when detecting 2 $\mu\text{g/kg}$ of ZEN in the positive sample extract (Figure 6C and D). The LOD of the B-FICS in real maize and wheat samples was defined as 2 $\mu\text{g/kg}$. The LOD of the A-FICS in maize and wheat samples was defined as 5 $\mu\text{g/kg}$ (Figure S4). In some actual situations, the concentrations of ZEN in the fungal contaminated cereal are always higher than the LOD of the developed sensors, so the sample extract needs to be further diluted to reach the detection limit of the sensors.

For the seven cereal samples in this study, semi-quantitative detection results obtained from both the B-FICS and the A-FICS were consistent with commercial ELISA kits (Table 2). In addition, the B-FICS has incomparable advantages in the detection sensitivity compared with the ELISA kit and A-FICSs (Table 3). This demonstrated that

Table 2: Comparison of the developed BP-Au nanocomposite-based fluorescent quenching immunochromatographic sensor (B-FICS), gold nanoparticles (AuNPs)-based fluorescence quenching immunochromatographic sensor (A-FICS) and the commercial ELISA kit for the detection of zearalenone (ZEN) in real cereal samples.

Sample	ELISA Average $\pm$ SD (n = 3) ($\mu\text{g/kg}$)	B-FICS (n = 3) ($\mu\text{g/kg}$)	A-FICS (n = 3) ($\mu\text{g/kg}$)
Maize 1	307.9 $\pm$ 9.9	+ ^a , +, +; 200 ~ 300 ^b	+, +, +; 250 ~ 500
Maize 2	ND ^c	- ^d , -, -; ND	-, -, -; ND
Maize 3	ND	-, -, - ^e ; ND	-, -, -; ND
Wheat 1	40.7 $\pm$ 5.3	+, +, +; 20 ~ 60	+, +, +; 25 ~ 50
Wheat 2	272.1 $\pm$ 37.8	+, +, +; 200 ~ 300	+, +, +; 250 ~ 500
Wheat 3	102.4 $\pm$ 12.4	+, +, +; 100 ~ 200	+, +, +; 100 ~ 250
Wheat 4	ND	-, -, -; ND	-, -, -; ND

^a+, positive results.

^b200 ~ 300, the concentration range of the ZEN which were determined by the FICS.

^cND, not detected.

^d-, negative results.

^e $\pm$, weakly positive results.

Table 3: Comparison of the analytical performance of the Au nanocomposite-based fluorescent quenching immunochromatographic sensor (B-FICS), gold nanoparticles-based fluorescence quenching immunochromatographic sensor (A-FICS) with the commercial ELISA kit in cereal samples.

	ELISA kit	B-FICS	A-FICS
Sample extraction time	15 min	10 min	10 min
Sample test time	>30 min	<20 min	<20 min
Result output form	Reading absorbances under microplate reader	Visual inspection under portable UV lamp	Visual inspection under portable UV lamp
Result measure time	5–10 min	On-site	On-site
LOD	7 µg/kg	2 µg/kg	5 µg/kg

LOD, limit of detection.

the B-FICS can be applied as a simple, rapid and sensitive detection method for ZEN sensing.

Declaration of competing interest: The authors declare no competing financial interest.

4 Conclusions

Due to the chemical instability of BPNSs, the BP-Au nanocomposite was directly synthesized in one step at room temperature, and the formation mechanism was explored by DFT calculation: the chemically unstable BPNSs reduced the Au^{3+} to Au atoms on the surface of the BPNSs, and the Au atoms aggregated to form AuNPs. The BP-Au nanocomposite can be applied as an excellent fluorescence quenching probe due to this high fluorescence quenching; the BP-Au nanocomposite can be simply synthesized at room temperature and the BP-Au nanocomposite provided sufficient physical adsorption sites for antibodies. The fluorescence quenching efficiency of the synthesized BP-Au nanocomposites to QDs ($\lambda_{\text{em}} = 525 \text{ nm}$) can reach 73.8%, which is 1.73-fold (42.6%) and 1.44-fold (51.2%) higher than pure AuNPs and BPNSs, respectively. The superior BP-Au-Ab was prepared and employed as an ideal immune fluorescence quencher probe to develop a B-FICS. The LOD of the developed B-FICS is 0.1 µg/l, which is 2.5-fold better than the traditional AuNPs-based FICS (A-FICS). In addition, the B-FICS can detect 2 µg/kg of ZEN in real cereal samples within 20 min. B-FICS offers a new and potentially effective application of BPNSs in food safety sensing. Also reported in this study was a novel method for rapid and high-sensitive detection of ZEN or other pollutants in foods.

Acknowledgments: This work was supported by “The National Key R&D Program of China” (No. 2016YFD0401202); and the Special Project of Tianjin Innovation Platform (No. 17PTGCCX00230).

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Supplementary Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/nanoph-2019-0434>).