

A sensitive immunosensor based on FRET between gold nanoparticles and InP/ZnS quantum dots for arginine kinase detection

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ABSTRACT

Arginine kinase (AK) is one of the most important allergens in shrimp products. Herein, a novel immunoassay for quantitation of AK was developed using the antibody modified gold nanoparticle (AuNP) and quantum dot (QD). When the first antibody modified AuNP (AuNP-Ab1) was bridged by AK with the secondary antibody modified QD (QD-Ab2), fluorescence resonance energy transfer (FRET) would occur between the AuNP and QD, which led to a decrease in fluorescent signals. The decrease in fluorescence intensity was found to correlate linearly with the log of AK concentration in the range of 1.0×10^{-6} – 1.0×10^{-3} mg/mL ($R^2 = 0.9909$) and the detection limit was 0.11 ng/mL. The immunoassay was further proved to have encouraging specificity, precision and accuracy. Compared with existing methods, this study provided a promising approach to develop a highly sensitive and selective detection method for AK in shrimp related food samples.

1. Introduction

Due to the improvement of people's living standards, food allergy has risen as a public concern over last decades (Sampson, 2016). Food allergy is known to trigger severe immunoglobulin E (Ig E)-mediated allergic reactions, such as atopic dermatitis, emesis, diarrhea, urticaria, angioedema, and may even endanger life (Sabra, Bellanti, Rais, Castro, de Inocencio, & Sabra, 2003). Shrimps and shrimp related products are popular among consumers because of their delicious taste, wide source and high nutrient content and economic value. However, as part of crustaceans, shrimps containing various allergenic proteins belong to the "Big eight" allergenic food types (milk, egg, fish, crustacean, tree nuts, peanut, soy and wheat) that cause severe food allergic reactions and are mandated for labelling on packaged foods in numerous countries such as the United States (U. S. Food And Drug Administration, 2004), the European Union (European Parliament and European Council, 2011) and Canada (Health Canada, 2011). Arginine kinase (AK) is found to be one of the most important allergens of shrimps and shrimp related products. It has certain thermal stability and its allergenicity is difficult to be eliminated through food processing (Giuffrida, Villalta, Mistrello, Amato, & Asero, 2014). Its molecular weight is about 40 kDa (Garcia-Orozco, Aispuro, Yepiz-Plascencia, Calderon de la Barca, & Sotelo-Mundo, 2007) and it has an isoelectric point of 6.19 (Yao, Ji, Kong, Wang, & Xiang, 2009). AK has regulatory and transport

functionalities, which plays an important role in the metabolism of invertebrates and catalyzes the production of ATP (Abdel Rahman, Kamath, Lopata, Robinson, & Helleur, 2011). AK exhibits a high homology within crustaceans such as shrimp (Khanaruksombat, Srisomsap, Chokchaichamnankit, Punyarit, & Phiriyangkul, 2014), crab (Shen et al., 2011) and mollusc (Shen et al., 2012), which could cause strong immune cross-reaction.

The existence of AK in shrimps and related products not only hinders the intake of delicious and protein-rich shrimp products by people with known allergies but also poses serious safety risks to people with unaware allergies and sometimes may even threatening their lives (Shen et al., 2012). Therefore, detection of AK in foods plays a pivotal role in avoiding unintended intake of allergenic foods for allergic people. Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) are the most widely employed detection methods for food allergens. For example, Lin et al. (2018) reported a validated ELISA for crustacean's major allergen tropomyosin (TM) with the limit of detection (LOD) as low as 0.030 ng/mL, exhibiting the ability in quantifying trace amount of the allergen in complex processed foods. However, for ELISA, the labeling process could be time-consuming and the multiple steps in the procedure limit improvement of the detection time (Wang et al., 2019)(Zeng et al., 2019). PCR-based methods were found to be able to detect 0.1 pg shrimp DNA and 0.0001% (w/w) of shrimp in model mixtures (Fernandes et al., 2018). However, PCR-based methods

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did not detect allergens directly, hence a difference between the detected results and actual allergen contents is often found (Herrero, Vieites, & Espiñeira, 2014). In recent years, fluorescent assays have featured fast developments for food allergens, which showed high sensitivity, specificity and easy operation requirements (Wang et al., 2019)(Chinnappan et al., 2019). For instance, a fluorescent aptamer biosensor was developed to exhibit a sensitive and selective detection ability of TM within 30 min (Chinnappan et al. 2019). However, fluorescence-based assays for AK are still quite limited at this stage.

Transfer of nonradiative energy from an excited donor to an acceptor by long-range dipole–dipole interactions has been known as fluorescence resonance energy transfer (FRET). Recently, bioanalysis based on FRET has gained particular interest (Liu, Freeman, Golub, & Willner, 2011; Wang, Mamedova, Kotov, Chen, & Studer, 2002). For traditional FRET systems, both the energy donor and acceptor are made of organic fluorescent dyes. However, these systems suffer from optical limitations such as spectral cross-talk, low resistance to chemicals and photobleaching, as well as small Stokes shifts, which makes the subsequent analysis complicate. It has been observed that nanostructures are highly promising for developing FRET systems due to the high efficiency of energy transfer (Rodzik-Czalka et al., 2018). For example, Wei et al. (2006) developed a sandwich immunosensor by employing an estrogen receptor β antigen (ER- β) monoclonal antibody modified 565 nm emitting quantum dot (QD) and Alexa Fluor 633 dye labeled anti-ER- β polyclonal reporter antibody. When the antigens were presented in the sample, a loss of QD fluorescence was found due to the formation of antibody-antigen-antibody structure and FRET between the QD and Alexa Fluor 633 dye. Besides, Oh et al. (2006) demonstrated that interaction of streptavidin-QD with biotinylated gold nanoparticle (AuNP) resulted in efficient quenching of QD fluorescence owing to the formation of QD-AuNP pairs and the subsequently occurred FRET. The quenching efficiency was higher than that measured with dye acceptors of similar structure (Oh, Hong, Lee, Nam, Yoon, & Kim, 2005). They also developed an inhibition assay for rapid detection of protein glycosylation based on the rate changes of FRET between carbohydrate-conjugated QD and lectin-conjugated AuNP (Oh, et al., 2006).

Quantum dot (QD) is nanoscale crystal with unique optical properties (Oh, et al., 2005). It has been extensively exploited as energy donor for FRET systems with many attractive features such as high quantum yields, broad absorption, large molar extinction coefficients, and narrow symmetric emission spectra (Huang & Ren, 2012). Gold nanoparticle (AuNP) is one category of the most widely used energy acceptors in constructing FRET systems because of the broad absorption spectra in the visible region that covers the emission wavelength range of common energy donors (Liu, Liang, Abdel-Halim, & Zhu, 2011). In addition,

AuNP is ready for labeling by antibodies via electrostatic interactions (Nie & Emory, 1997). Herein, we developed a novel immunosensor for AK by employing the antibody modified AuNP (AuNP-Ab1) and InP/ZnS QD (QD-Ab2). When the AuNP-Ab1 and QD-Ab2 were bridged together by the AK in the sample, the distance between the QD and AuNP was supposed to be greatly shortened, which led to FRET and a decrease in fluorescent signal of QD (Fig. 1). The standard curve, detection limit, specificity, precision and accuracy of the method were carefully studied and its applicability in real shrimp samples was also validated. It's believed that the proposed immunoassay would offer a promising approach for food allergen detection.

2. Material and methods

2.1. Reagents and materials

3.5 nm InP/ZnS QD (InP is the core and ZnS is the shell, and the surface of QD is presented with carboxyl groups) was purchased from Xingzi New Material Technology Development Co., Ltd (Shanghai, China). The average quantum yield of the QD is 50%. AK monoclonal antibodies (Ab1 and Ab2) were purchased from Jieshengjiekang Biotechnology Co., Ltd (Wuxi, China). Chloroauric acid (HAuCl₄), bovine serum albumin (BSA), ovalbumin (OVA) and β -lactoglobulin (β -LG) were purchased from Sigma-Aldrich (Shanghai, China). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and all other chemical reagents in analytical grade were purchased from Aladdin (Shanghai, China). Tropomyosin (TM) was prepared by our laboratory following the previous work (Wang, Rao, Zhou, Zheng, & Fu, 2019). Soybean protein isolation (SPI) derived from defatted soy meal was purchased from Wanbang Industrial Co., Ltd. (Henan, China). Representative crustaceans (*Pleoticus muelleri*, *Penaeus vannamei*, *Fenneropenaeus chinensis*, *Macrobrachium nipponense*, *Trachypenaeus curvirostris*, *Portunus trituberculatus*, *Procambarus clarkia*, *Crassostrea gigas*), processed shrimp products (shrimp dumplings #1, shrimp paste, prawn ball, dried small shrimps, hot pot flavored shrimp dumplings and shrimp dumplings #2) and typical food materials (almond, wheat, beef, mutton and chicken) were purchased from a local supermarket (Hangzhou, China). Plant and animal total protein extraction kits were purchased from Sangon Biotech (Shanghai, China). Whole cell lysis kit and Bicinchoninic acid (BCA) protein quantitation assay kit were bought from Nanjing KeyGen Biotech. (Nanjing, China). ELISA plate was purchased from Shanghai Wolhong Biology Company (Shanghai, China). All solution was prepared using ultrapure water (18.2 M Ω) purified by a Milli-Q system (Millipore, Massachusetts, USA).

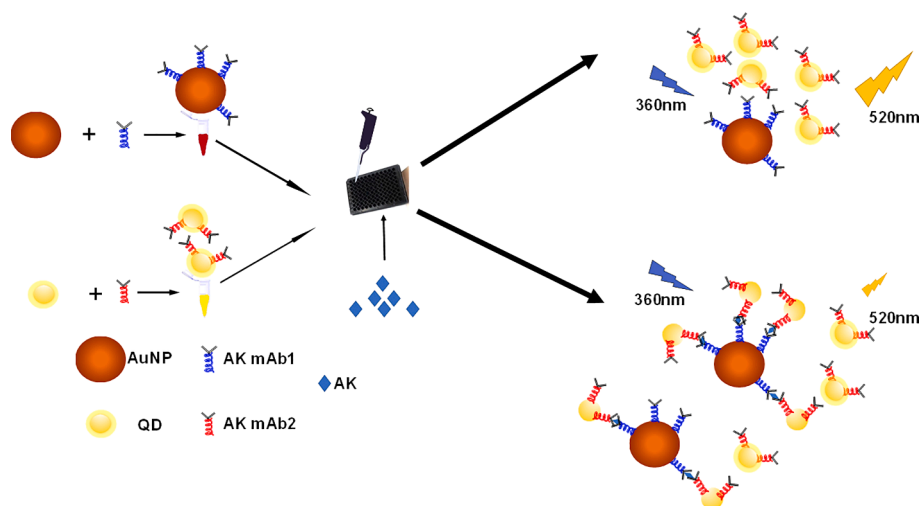


Fig. 1. Schematic illustration of the proposed immunosensor based on FRET between antibody modified AuNP and InP/ZnS QD for AK detection.

2.2. Preparation of the monoclonal antibody modified AuNP (AuNP-Ab1)

2.2.1. Synthesis and characterization of AuNP

All glassware was cleaned thoroughly by aqua regia (3:1 HCl/HNO₃) and then rinsed extensively with Milli-Q water. AuNP was synthesized by reduction of HAuCl₄ through trisodium citrate according to the previous study (Wang, Rao, Zhou, Zheng, & Fu, 2019). Briefly, a 48.75 mL Milli-Q water and 0.6 mL of HAuCl₄ (4 g/mL) were added in a round bottom flask. The solution was heated to 160 °C under vigorous stirring. Then 1.4 mL of freshly prepared 1% (w/v) trisodium citrate solution was added to the boiling HAuCl₄ solution. The solution color gradually turned to wine red within 5 min. The gold colloids were cooled to room temperature under stirring.

2.2.2. Modification of AuNP with monoclonal antibody Ab1

The AuNP-Ab1 was synthesized by direct adsorption of first antibody (Ab1) onto AuNP as the previously reported strategy (Ciaurriz, Fernandez, Tellechea, Moran, & Cabrera Asensio, 2017). This strategy relied on electrostatic, hydrophobic, and hydrophilic interactions between Ab1 and the surface of AuNP (Shi, Yuan, Wu, & Liu, 2011). Briefly, 10 µL of Ab1 (50 µg/mL) was added to 1 mL of as-prepared AuNP suspension (pH 8.5, adjusted by 0.1 M K₂CO₃). Then the mixture was incubated at room temperature for 1 h. Next, 10 µL BSA (500 µg/mL) was added to the above mixture and continued to incubate at room temperature for 1 h to block the nonspecific binding sites. The conjugates were centrifuged at 13,000 g for 20 min and this step was repeated 3 times to remove the unbound Ab1. The Ab1 labeled AuNP (AuNP-Ab1) was stored in Tris-HCl (0.01 mM, pH = 7.52) at 4 °C.

2.3. Preparation of the monoclonal antibody modified InP/ZnS QD (QD-Ab2)

The secondary AK monoclonal antibody (Ab2) was conjugated to COOH-functionalized InP/ZnS QD nanoparticle by EDC chemistry (Kim, Ahn, Moon, Park, Lee, & Choi, 2014). Briefly, 100 µL QD (5 mg/mL) was mixed with 100 µL 0.005 mg/mL of Ab2 and 10 µL 10 mg/mL of EDC. The incubation was carried out in dark for 2 h shaking at room temperature. The excess EDC was removed through 3 times ultrafiltration (3990 g, 20 min). The purified QD-Ab2 probes were stored at 4 °C.

2.4. Characterization

The morphologies of the AuNP and InP/ZnS QD before and after antibody modification were analyzed by transmission electron microscope (TEM, FEI Tecnai G2 F20, FEI, USA). The hydrodynamic size of the nanoparticles was studied by Zetasizer (Zetasizer Nano ZS90, Malvern, UK).

2.5. Standard curve for the proposed immunoassay

0.05 mL of AuNP-Ab1 (the concentrations of Ab1 was 2.5 µg/mL) probes and 0.05 mL of AK standard samples (the concentrations of AK were 1, 5, 10, 50, 100, 500, and 1000 ng/mL, respectively) were added in 96-well black plates and shaken for 10 min. Then, 0.05 mL of QD-Ab2 (the concentration of Ab2 was 2.5 µg/mL) probes were added into the mixture of AuNP-Ab1 and AK and allowed to interact for 30 min. The fluorescence signal was measured by a microplate reader (Molecular Devices i3x + MinMax). The excitation wavelength was 360 nm and the emission signal at 520 nm was collected.

2.6. Specificity of the proposed immunoassay

TM and BSA were used along with AK to study the specificity of the proposed method. Briefly, 0.05 mL of AuNP-Ab1 probes and 0.05 mL of AK standard samples (the concentration was 1000 ng/mL) were added

in 96-well black plates and shaken for 10 min. Then 0.05 mL of QD-Ab2 probes were added into the mixture of AuNP-Ab1 and AK and allowed to interact for 30 min. The fluorescence signal was measured by a microplate reader immediately. The excitation wavelength was 360 nm, and the emission wavelength was 520 nm.

2.7. Precision of the proposed immunoassay

The precision of the detection method was assessed by the intra-day and inter-day assay at different concentrations (10, 20, 30 ng/mL) of AK. The following steps were the same as Section 2.5.

2.8. Recovery of the proposed immunoassay

AK was spiked in three different levels (10 ng/mL, 20 ng/mL and 30 ng/mL), and then subjected to the procedures described in Section 2.5. Two kinds of samples, blank samples (pure water) and real shrimp samples, were used for the recovery analysis. For real shrimp samples, the AK content was firstly measured and then the spiked AK (1.0 ng, 2.0 ng and 3.0 ng) was further analyzed.

2.9. Shrimp sample preparation and analysis

The total protein of the six representative species of commercial shrimps was extracted by whole cell lysis kit. The total protein content was determined by BCA protein quantitation assay and further diluted 10,000 times by Tris-HCl. The AK contents in the six shrimp samples were measured by the proposed immunoassay.

2.10. Shrimp processed product sample preparation and analysis

The samples of the six representative commercial shrimp products, that were shrimp dumplings #1 (thermally treated), shrimp paste, prawn ball (thermally treated), dried small shrimps, hot pot flavored shrimp dumplings (thermally treated) and shrimp dumplings #2 (containing large shrimp meat), were prepared using whole cell lysis kit and AK contents were determined via immunosensor as Section 2.9. Meanwhile, the AK contents were also determined by ELISA. Briefly, 100 µL pure AK of different concentrations and six shrimp processed product samples were added to 96-well microtiter plate for immobilization at 4 °C overnight. Next, the ELISA plate was washed three times and added with 200 µL 5% w/w BSA solution to block for 2 h. After blocking, the plate was washed 3 times and incubated with 100 µL 0.2 µg/mL Ab1 at 37 °C for 1.5 h. Next, the plate was washed 3 times and incubated with 100 µL 0.2 µg/mL horseradish peroxidase (HRP)-labeled goat anti-mouse IgG (Abcam, China) at 37 °C for 1.5 h. After incubation, the plate was washed 3 times and added with 100 µL of tetramethylbenzidine (TMB) solution following with incubation at 37 °C in avoidance of light for 20 min. Finally, the reaction was stopped by addition of 50 µL 2 M H₂SO₄ and the absorbance at OD 450 nm was measured. The AK contents were then back-calculated from the standard curve of the ELISA and compared with those measured by the immunosensor.

2.11. Cross-reactivity

Total protein extracts from the plant (almond and wheat) and animal (beef, mutton, chicken and fish) species that commonly used as food were obtained by following the standard procedures of the plant and animal total protein extraction kit. The cross-reactivity of Ab1 to AK among different species of crustaceans, nontarget allergens such as ovalbumin (OVA), tropomyosin (TM) and β-lactoglobulin (β-LG), protein isolation such as soybean protein isolation (SPI) and total protein extracts from the common plant and animal species was carefully studied using the ELISA method described in Section 2.10.

3. Results and discussion

3.1. Preparation of first antibody modified AuNP (AuNP-Ab1)

AuNP was synthesized according to the method reported by (Wang et al., 2019). The Ab1 was directly adsorbed onto the surface of AuNP as Fig. S1A showed. The TEM images of the AuNP and AuNP-Ab1 were shown in Fig. S2. It could be found out that the AuNP and AuNP-Ab1 were round-shaped and well-dispersed. The UV-Vis spectra of the AuNP and AuNP-Ab1 showed that the adsorption peak of the AuNP was at 515 nm while the adsorption peak of the AuNP-Ab1 was at 523 nm (Fig. 2A). The red-shift in adsorption spectra proved that the Ab1 was successfully adsorbed onto the AuNP's surface (Shi, et al., 2011). The DLS results showed that the AuNP particles were well-dispersed with an average hydrodynamic size of 30.52 ± 0.47 nm (Fig. 2B). After modification of Ab1, the hydrodynamic size of AuNP dramatically increased to 46.96 ± 0.16 nm (Fig. 2B), indicating the successful adsorption of Ab1 on AuNP.

The ratio between AuNP and Ab1 for AuNP-Ab1 preparation was optimized by studying the $OD_{520}-OD_{580}$ of AuNP-Ab1, which represented the stability of the AuNP (Sun, Zhao, Tang, & Jun 2005). That is, AuNP with higher $OD_{520}-OD_{580}$ had better stability than those with lower $OD_{520}-OD_{580}$ and vice versa. As Fig. 2C showed, the $OD_{520}-OD_{580}$ gradually increased and reached the highest when the concentration of Ab1 was 50 $\mu\text{g/mL}$. So, the optimum concentration of Ab1 was chosen to be 50 $\mu\text{g/mL}$.

3.2. Preparation of secondary antibody modified InP/ZnS QD (QD-Ab2)

The Ab2 was conjugated onto QD by EDC chemistry as Fig. S1B showed. The TEM images of the QD and QD-Ab2 shown in Fig. S3 indicated that the QD and QD-Ab2 were round-shaped and well-dispersed. The fluorescence spectra in Fig. 2D showed that the emission peak was 520 nm for the QD and 530 nm for QD-Ab2 probes. As the conjugation of antibody onto QD could result in a red-shift in emission spectra (Kim et al., 2017), this suggested that the Ab2 was successfully conjugated onto QD. The lower fluorescence intensity of QD-Ab2 than that of QD could be a consequence of the quenching effect of the coupling agent (Wang, Bai, & Wei, 2011).

The ratio between Ab2 to QD was also carefully optimized. QD with concentration in the range of 1–9 mg/mL was used to react with the same amount of Ab2. As shown in Fig. 2E, the conjugation efficiency of Ab2 reached a plateau at a concentration above 5 mg/mL of QD. Herein,

5 mg/mL was chosen to be the optimum concentration of QD for Ab2 conjugation. As Fig. S4 shows, there was an overlap between the emission spectrum of QD-Ab2 and the absorption spectrum of AuNP-Ab1 around 520 nm, indicating that the QD-Ab2 and AuNP-Ab1 could work as a donor-acceptor pair to exert FRET.

3.3. Optimization of the detection condition

To ensure the adequate interaction between the complex of AuNP-Ab1 and AK (SDS-PAGE image of AK is shown in Fig. S5) with QD-Ab2, different detection time of 10, 20, 30, 40, 50 and 60 min was studied to optimize the detection condition. As shown in Fig. S6, the decrease in fluorescence signal almost remained unchanged for detection time longer than 30 min. Therefore, 30 min was chosen as the optimum detection time for the proposed immunoassay.

3.4. Method validation of the proposed immunoassay

AK standards in nine different concentration (1, 5, 10, 50, 100, 500 and 1000 ng/mL) were used to obtain the standard curve of the proposed immunoassay. It could be seen from Fig. 3A that the fluorescence signals decreased linearly with the increment of the log of the AK concentration in a range of 1×10^{-6} to 1×10^{-3} mg/mL. The standard curve was determined to be $Y = -0.117 \cdot X - 0.2381$ with the correlation coefficient (R^2) being 0.9909, where Y was F (fluorescence intensity $E_{520 \text{ nm}}$ of the sample)/ F_0 (fluorescence intensity $E_{520 \text{ nm}}$ of the blank QD sample) and X was log of the AK concentration (mg/mL). When AK bridged the AuNP-Ab1 and QD-Ab2 together, the distance between AuNP-Ab1 and QD-Ab2 would decrease to lead to a FRET between AuNP-Ab1 and QD-Ab2, which would result in decreased fluorescent signals. That is, the fluorescent signal of QD-Ab2 quenched by AuNP-Ab1 would increase as a function of the concentration of AK. The corresponding limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 0.11 ng/mL ($S/N = 3$) and 9.4 ng/mL ($S/N = 10$), respectively. Therefore, the proposed immunoassay exhibited improved sensitivity in comparison with the ion-exchange chromatography coupled with dynamic coating capillary electrophoresis method (LOD = 1.2 $\mu\text{g/mL}$) for AK determination reported by Fu et al. (2018). Compared with the PCR-based method that was able to detect 0.1 pg shrimp DNA and covered the detection range of 0.0001–50% (w/w) of shrimp in model mixtures (Fernandes et al., 2018), the proposed immunosensor detected the allergen directly and was more suitable for quantifying AK at trace amounts due to the high sensitivity below 0.0001% (w/w) of AK

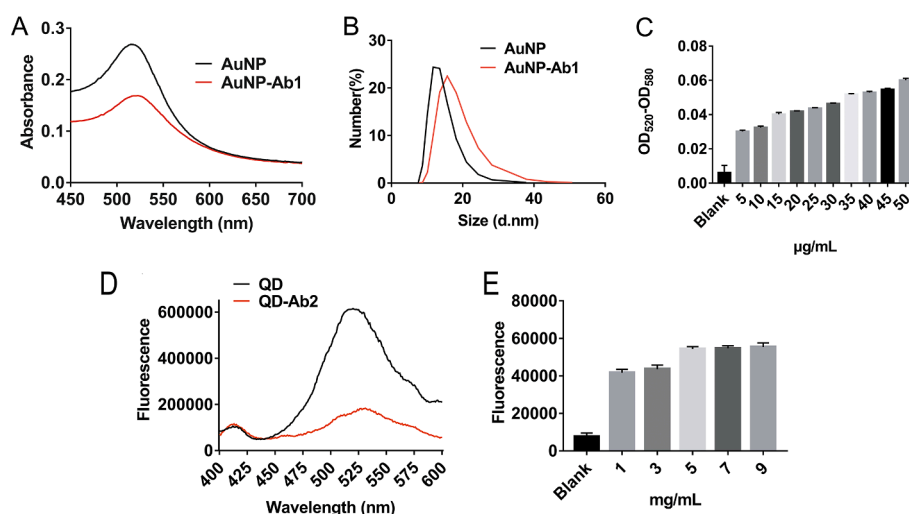


Fig. 2. (A) UV-Vis spectra of the AuNP (black line) and AuNP-Ab1 (red line). (B) Size distribution of the AuNP (black line) and AuNP-Ab1 (red line). (C) Optimization of the Ab1 modification on the AuNP's surface. (D) Fluorescence spectra of the QD (black line) and QD-Ab2 (red line). (E) Optimization of the Ab2 modification on the QD's surface. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

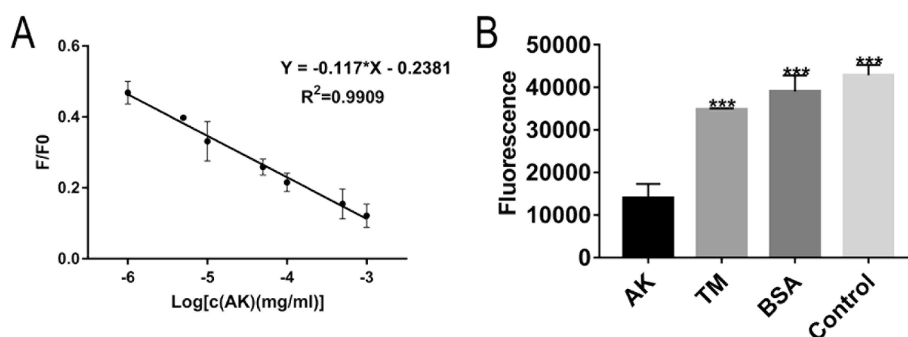


Fig. 3. (A) Standard curve of the proposed immunoassay for AK detection ($n = 3$). (B) Specificity of the proposed immunoassay. BSA, TM were chosen as model nonspecific targets. (Statistical analysis was conducted by Student's t test compared with AK. *** represented $p < 0.001$.)

in the samples. For samples with high AK concentration, extra dilution procedures would be needed. In addition, compared with the chiral immunosensor and surface plasmon resonance immunosensor we developed previously for tropomyosin determination (Wang, Rao, Zhou, Zheng, & Fu, 2019; Zhou et al., 2019), the proposed immunoassay did not require specific instruments to analyze the chiral or surface plasmon resonance signals, thus was potential for wider applications.

To verify the specificity of this assay, two possible interfering components, TM (one of the major allergens in shrimp) and BSA (albumin from bovine serum), were used as model nonspecific targets. The samples containing the same amount of AuNP-Ab1 and QD-Ab2 were chosen as control samples. As shown in Fig. 3B, the fluorescence intensities for BSA and TM were roughly the same as the control samples, while AK resulted in a tremendous decrease in fluorescence intensity. These results suggested that the developed immunoassay possessed excellent specificity to AK.

The precision of the detection was also assessed by the intra-day and inter-day assay with different concentrations (10, 20, 30 ng/mL) of AK. As could be seen from Table 1, the CV% of intra-day assay was 4.60%–8.65% and inter-day assay was 4.26%–7.51%. These results indicated that the proposed immunoassay had good precision and repeatability.

The recovery rates of the proposed immunoassay were analyzed by blank samples and real shrimp samples spiked with AK standards. The results showed that the recoveries were in the range of 89.04%–119.65% and 90.14% to 105.13% for the spiked blank samples (Table 2) and real shrimp samples (Table S1), suggesting that the proposed method had good accuracy.

3.5. Detection of AK in shrimp samples

To validate the performance of the proposed immunoassay in real shrimp samples. The proposed immunoassay was further used to detect the AK in six kinds of shrimps to validate the feasibility of the method for real-world application. The SDS-PAGE image in Fig. S7 proved that the total proteins of the six shrimps were successfully extracted. The AK contents in the diluted samples from different shrimp species measured by the proposed method were shown in Table 3. Compared with the AK contents measured for *Fenneropenaeus chinensis* (2.62 mg/g) and *Macrobranchium nipponense* (1.53 mg/g) by the capillary electrophoresis-

Table 1
Precision of the proposed immunoassay in AK spiked samples ($n = 3$).

	Spiked concentration (ng/mL)	Measured AK Conc. $\pm$ SD (ng/mL)	CV (%)
Intra-day	10	10.52 $\pm$ 0.91	8.65
	20	17.68 $\pm$ 1.20	6.81
	30	29.00 $\pm$ 1.33	4.6
Inter-day	10	9.16 $\pm$ 0.39	4.26
	20	18.39 $\pm$ 0.81	4.50
	30	32.10 $\pm$ 2.41	7.51

Table 2
Recovery rates of the proposed immunoassay in AK spiked blank samples ($n = 3$).

Sample No.	Spiked concentration (ng/mL)	Measured AK Conc. $\pm$ SD (ng/mL)	Recovery (%)
1	10	10.83 $\pm$ 1.56	108.31
	20	23.93 $\pm$ 2.03	119.65
	30	33.83 $\pm$ 3.42	112.77
2	10	8.90 $\pm$ 0.47	89.04
	20	22.61 $\pm$ 5.97	113.05
	30	29.01 $\pm$ 4.09	96.71
3	10	9.56 $\pm$ 0.89	95.60
	20	19.59 $\pm$ 2.83	97.95
	30	31.22 $\pm$ 4.65	106.07

Table 3
AK contents in six kinds of shrimps detected by the immunoassay ($n = 3$).

Shrimp species	Measured AK Conc. $\pm$ SD (mg/g)	Percentage of shrimp protein (%)
<i>Pleoticus muelleri</i>	2.46 $\pm$ 0.23	13.69
<i>Penaeus vannamei</i>	1.09 $\pm$ 0.12	6.82
<i>Fenneropenaeus chinensis</i>	3.08 $\pm$ 0.29	12.73
<i>Macrobranchium nipponense</i>	1.62 $\pm$ 0.11	7.69
<i>Trachypenaeus curvirostris</i>	2.87 $\pm$ 0.25	14.87
<i>Penaeus monodon</i>	1.13 $\pm$ 0.10	13.69

based method in the previously reported work (Fu et al. 2018), the proposed immunosensor gave out comparable results. The results demonstrated the feasibility of the proposed immunoassay for AK detection in real-world shrimp samples.

3.6. Detection of AK in shrimp processed products

The proposed immunoassay was further employed to quantify AK contents in real shrimp processed food samples that were shrimp dumplings #1, shrimp paste, prawn ball, dried small shrimps, hot pot flavored shrimp dumplings and shrimp dumplings #2. The SDS-PAGE image in Fig. S8 proved that the total proteins of the six shrimp products were successfully extracted. The AK contents in all of the shrimp processed product samples were successfully determined by the immunosensor (Table S2), while the shrimp dumplings #1 and prawn ball only showed positive signals below LOQ in the results of ELISA due to the relatively low AK amounts. For shrimp paste, dried small shrimps, hot pot flavored shrimp dumplings and shrimp dumplings #2, the AK contents measured by the immunosensor were consistent with these determined by ELISA (back-calculated from the standard curve in Fig. S9). These results indicated that the immunosensor was feasible for AK detection in real-world shrimp products, even those with relatively

low contents of AK. Moreover, the proposed immunosensor did not need labelling, contrarily to ELISA (Wang et al., 2019)(Zeng et al., 2019), having significantly shortened time of analysis (<1 h).

In addition, the cross-reactivity of Ab1 (the recognition element of the immunosensor) to the AK from different crustacean and shellfish species and non-target proteins was carefully studied. Fig. S10 showed that the Ab1 had relatively high affinity to shrimp (*Penaeus vannamei*), crayfish (*Procambarus clarkii*) and oyster (*Crassostrea gigas*), while had moderate affinity to crab (*Portunus trituberculatus*). Therefore, the proposed immunosensor could potentially extend to detect AK of the crustacean species that share high homology with shrimp. Moreover, as Fig. S12 showed, the Ab1 exhibited negligible affinity to non-target allergens such as ovalbumin (egg white), tropomyosin (another major allergen of crustacean) and β -lactoglobulin (milk). At the same time, no significant cross-reactivity was found against the isolated proteins of commonly used food species such as soybean, almond, wheat, beef, mutton, chicken and fish (SDS-PAGE image in Fig. S11 indicated the successful extraction of the total proteins). Therefore, we suggested that the proposed immunosensor could be used for detecting AK in processed food products and could merit for identification of potential adulterations of shrimp or related crustacean species in seafood products.

4. Conclusions

In summary, a novel immunosensor was demonstrated to be able to detect AK by taking advantage of the FRET between antibody modified AuNP and QD. The proposed immunoassay showed a standard curve of $Y = -0.117 \times X - 0.2381$ in the range of 1.0×10^{-6} – 10^{-3} mg/mL. The detection limit was further determined to be 0.11 ng/mL. Moreover, good precision of this immunoassay was ensured by the CV (%) of intra-day and inter-day as 4.60%–8.65% and 4.26%–7.51%. The recoveries were found to range from 89.04% to 119.65% and 90.14% to 105.13% in the spiked blank samples and real shrimp samples. Besides, the method was able to detect AK in six shrimp samples and six processed food samples. These results suggested that the immunoassay was a reliable and sensitive method for the detection of AK and offered a simple yet promising approach for food allergen detection.

CRediT authorship contribution statement

Yanbo Wang: Conceptualization, Methodology, Supervision. **Junjie Ma:** Investigation, Data curation, Writing - original draft. **Huan Li:** Writing - review & editing, Visualization. **Jinru Zhou:** Methodology, Validation. **Hong Zhang:** Resources, Visualization. **Linglin Fu:** Conceptualization, Supervision, Funding acquisition.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2021.129536>.

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**A sensitive immunosensor based on FRET between gold nanoparticles and
InP/ZnS quantum dots for arginine kinase detection**

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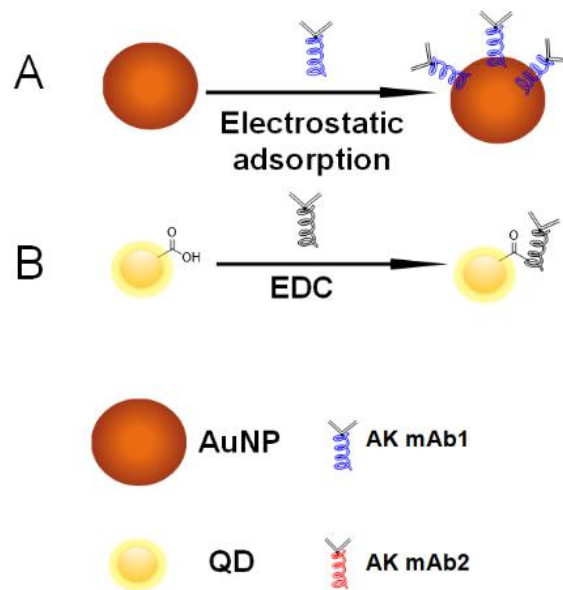


Figure S1. Schematics of the preparation procedures for the (A) AuNP-Ab1. (B) QD-Ab2.

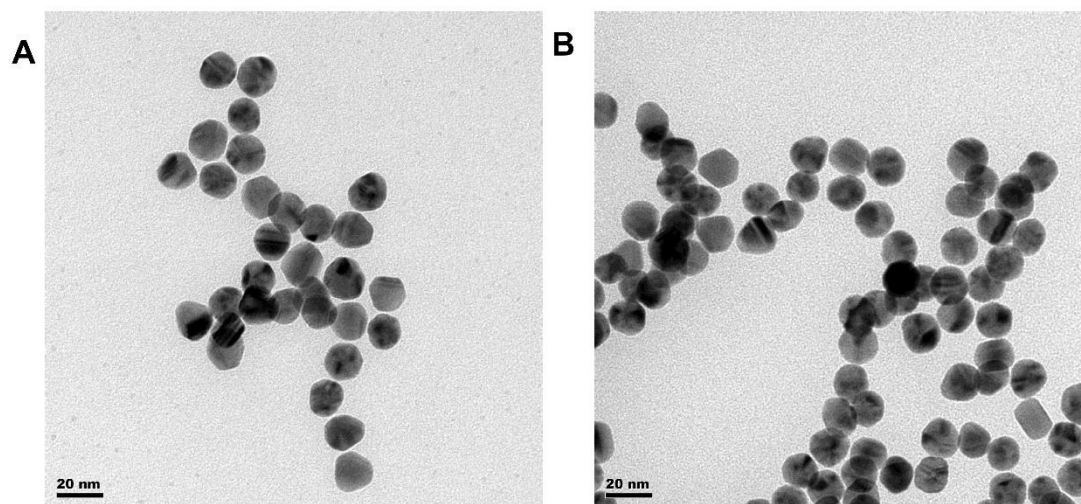


Figure S2. TEM images of the (A) AuNP and (B) AuNP-Ab1.

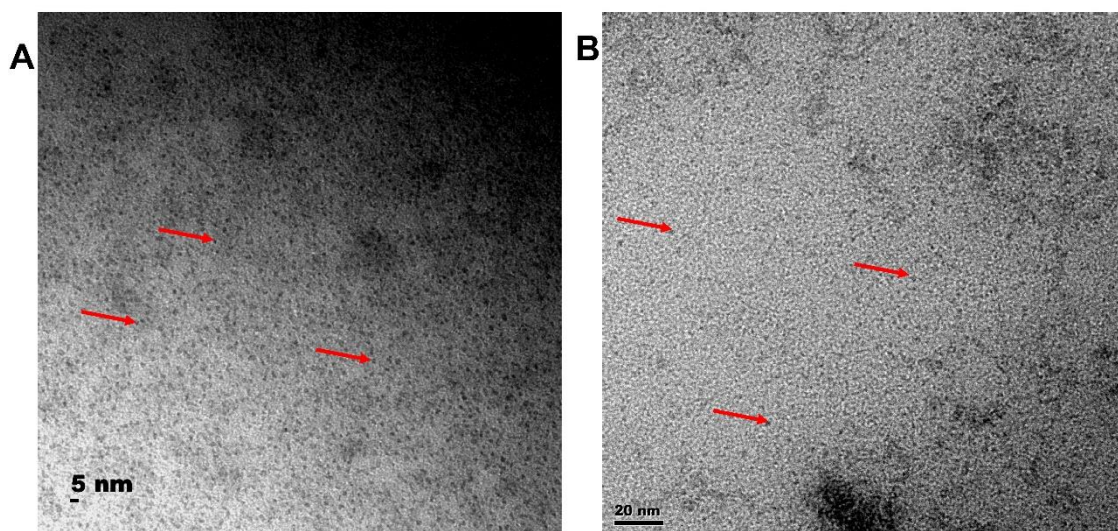


Figure S3. TEM images of the (A) QD and (B) QD-Ab2. (Red arrows indicate QD)

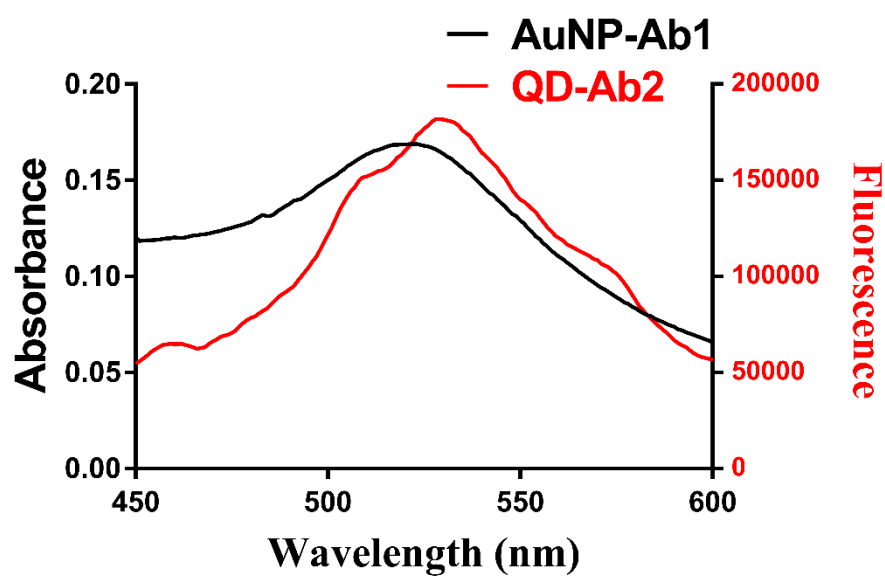


Figure S4. The spectral overlap between the absorption spectrum of AuNP-Ab1 and emission spectrum of QD-Ab2.

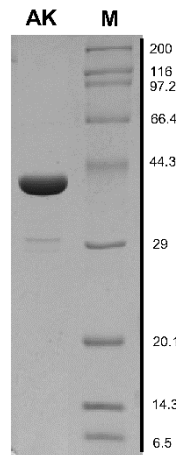


Figure S5. SDS-PAGE of AK.

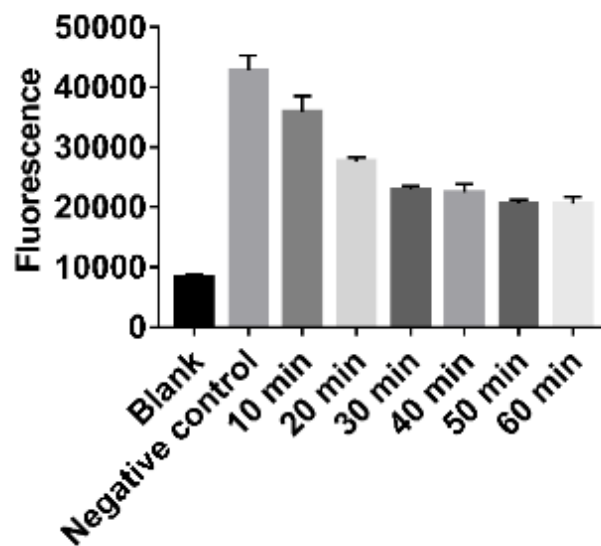


Figure S6. Optimization of the incubation time for the immunoassay.

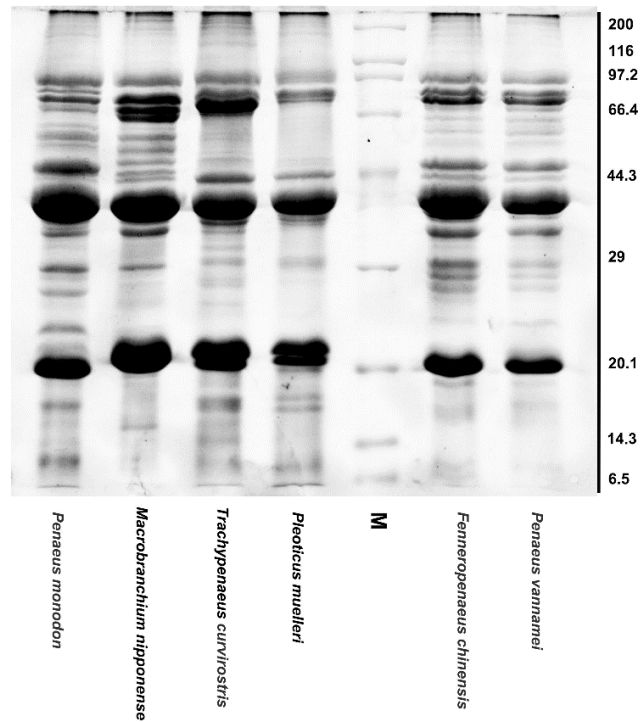


Figure S7. SDS-PAGE of the total protein extracts from six kinds of shrimps (*Fenneropenaeus chinensis*, *Macrobranchium nipponense*, *Penaeus vannamei*, *Penaeus monodon*, *Trachypenaeus curvirostris*, *Pleoticus muelleri*).

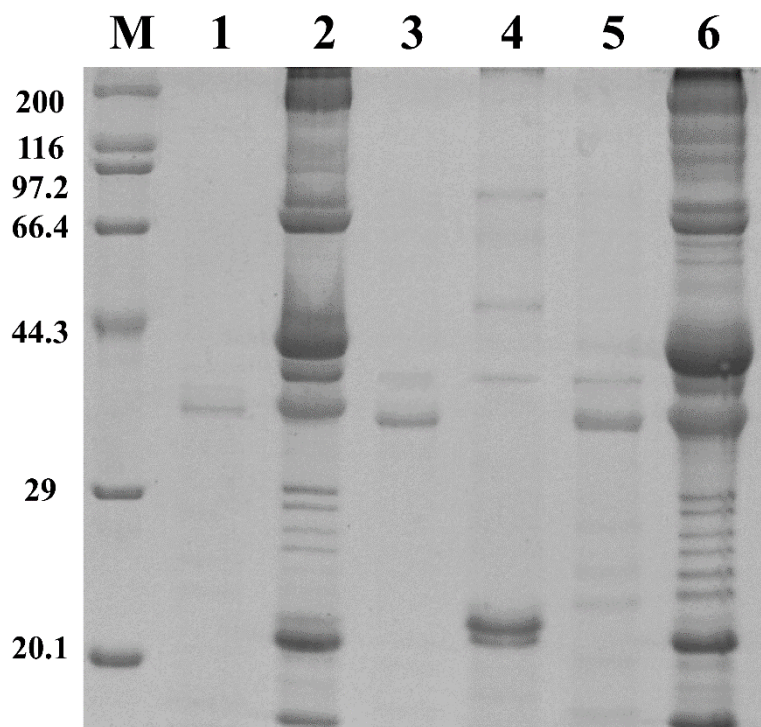


Figure S8. SDS-PAGE of the total protein extracts from shrimp processed

products (M: marker, 1: shrimp dumplings #1, 2: shrimp paste, 3: prawn ball, 4: dried small shrimps, 5: hot pot flavored shrimp dumplings, 6: shrimp dumplings #2).

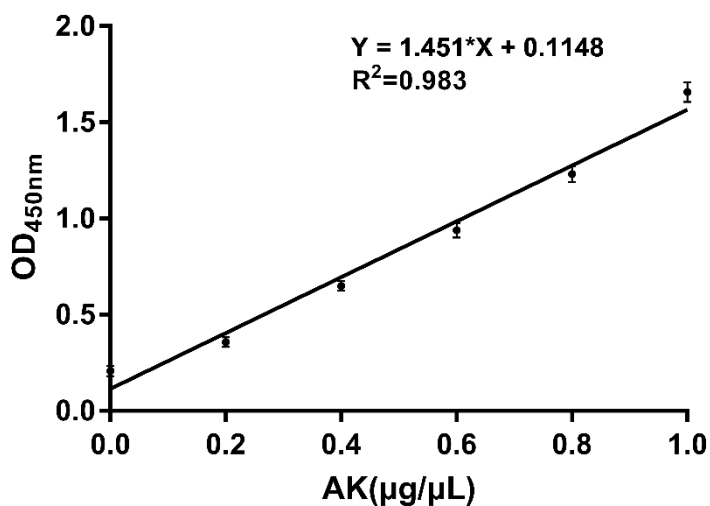


Figure S9. Standard curve of ELISA for AK detection (n=3).

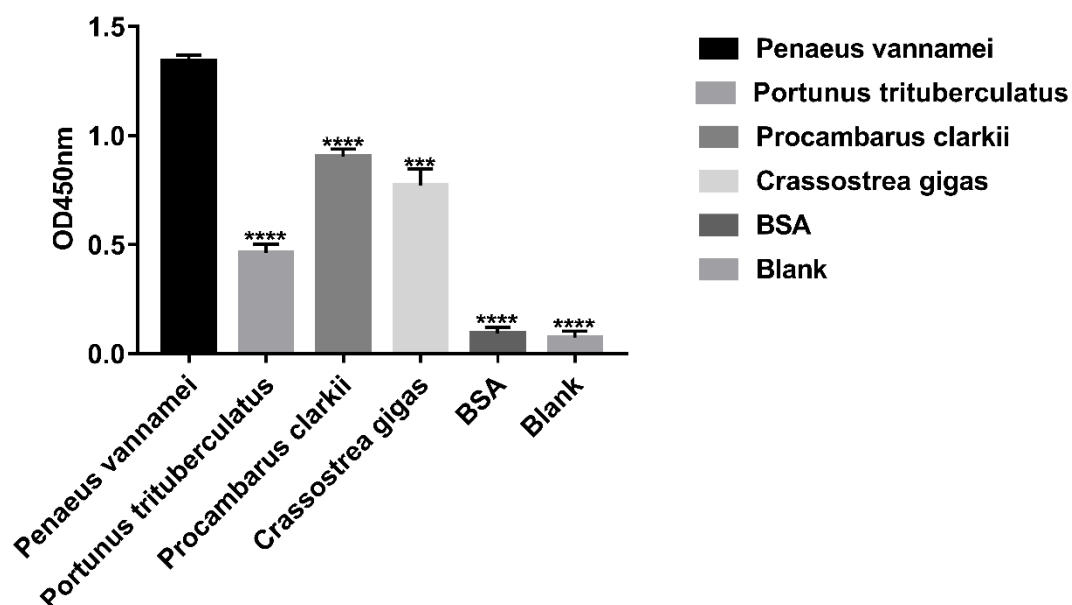


Figure S10. The cross-reactivity of Ab1 to AK from different seafoods (n=3).

(Statistical analysis was conducted by Student's t test compared with AK. ***

represented $p < 0.001$. **** represented $p < 0.0001$)

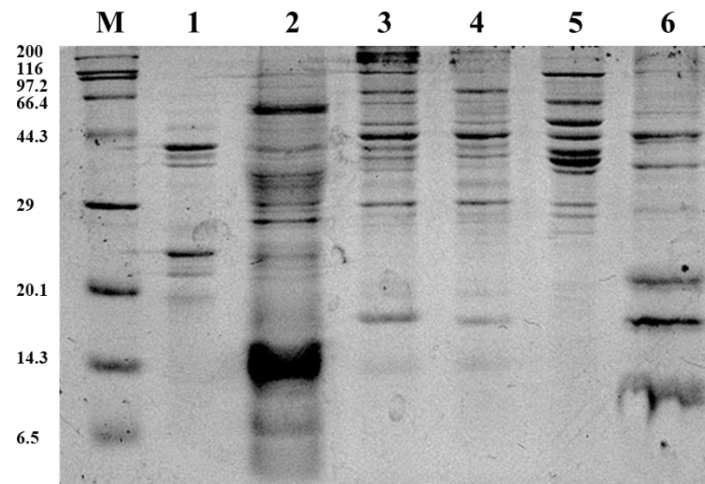


Figure S11. SDS-PAGE of the total protein extracts from the plant and animal species that commonly used as food (M: marker, 1: almond, 2: wheat, 3: beef, 4: mutton, 5: chicken 6: fish)

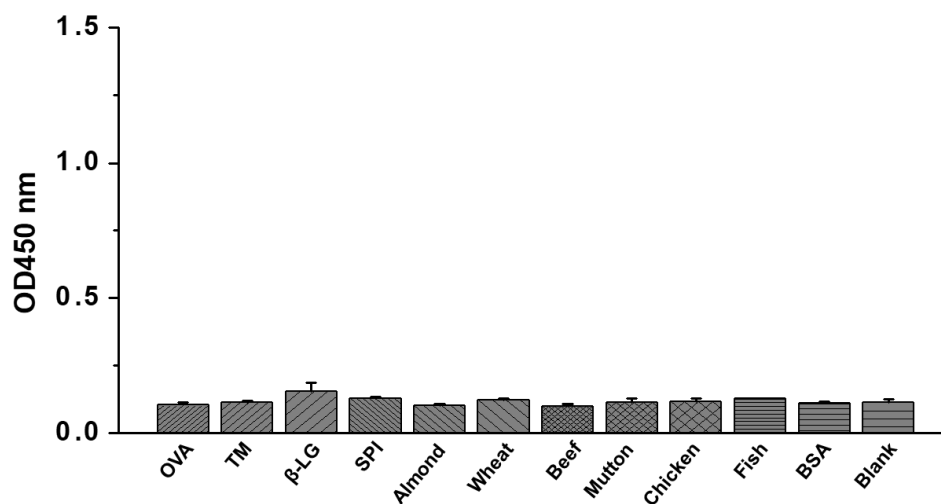


Figure S12. The cross-reactivity of Ab1 to nonspecific allergens such as ovalbumin (OVA), tropomyosin (TM) and β -lactoglobulin (β -LG), protein isolation such as soybean protein isolation (SPI) and total protein extracts from common plant and animal species such as almond, wheat, beef, mutton, chicken and fish.

Table S1. Recovery rates of the proposed immunoassay in AK spiked real shrimp samples (n=3).

Real shrimp content of AK (mg/g)	Spiked amount (ng)	Measured AK Content±SD (ng)	Recovery (%)
0.18	1.0	1.02±0.10	102.19
0.17	1.0	0.99±0.08	98.61
0.19	1.0	0.98±0.10	97.93
0.36	2.0	1.94±0.25	97.35
0.39	2.0	1.84±0.26	94.73
0.35	2.0	1.80±0.19	90.14
0.54	3.0	2.93±0.39	97.73
0.59	3.0	3.15±0.34	105.13
0.53	3.0	2.79±0.49	93.23

Table S2. AK contents in six kinds of shrimp products detected by the immunoassay (n=3).

Shrimp products	Immunosensor Measured	ELISA Measured AK
	AK Conc.± SD (mg/g)	Conc.± SD (mg/g)
Shrimp dumplings #1	0.39±0.13	Positive but below the LOQ
Shrimp paste	2.91±0.36	2.76±0.27
Prawn ball	0.27±0.10	Positive but below the LOQ
Dried small shrimps	1.14±0.19	1.35±0.11
Hot pot flavored shrimp dumplings	1.51±0.16	1.83±0.19
Shrimp dumplings #2	3.02±0.59	2.91±0.22