



Quantification of shellfish major allergen tropomyosin by SPR biosensor with gold patterned Biochips

Jinru Zhou^{a,b}, Yanbo Wang^b, Yifan Qian^b, Teng Zhang^c, Lei Zheng^{a,**}, Linglin Fu^{b,*}

^a School of Food Science and Engineering, Hefei University of Technology, Hefei, 230009, China

^b Zhejiang Engineering Institute of Food Quality and Safety, Zhejiang Gongshang University, Hangzhou, 310018, China

^c ZhongLongYiCheng Technology Company, Beijing, 100166, China

ARTICLE INFO

Keywords:

Shellfish allergen
Tropomyosin
SPR
Gold chip
Food safety

ABSTRACT

Tropomyosin (TM), known as the major allergen in seafood, has been increasing concerns in industrialized countries. We proposed a simple, rapid and accurate analytical method using surface plasmon resonance (SPR) sensor with gold patterned biochips for detection and quantification of TM in shellfish. The limit of detection (LOD) was $1.0 \mu\text{g mL}^{-1}$ ($S/N = 3$), while the limit of quantitation (LOQ) was $2.5 \mu\text{g mL}^{-1}$ ($S/N = 10$). Moreover, the satisfactory recoveries (92.0–108.0%) was obtained for TM detection in food samples, showing the good accuracy of this method. Furthermore, the results also demonstrated that this developed method is suitable for the determination of TM from different shellfish species within 3 min. Therefore, the developed SPR sensor with gold patterned biochips will have positive significance on the rapid and accurate detection allergens in shellfish foodstuffs, related products and their production lines in the future.

1. Introduction

Food allergy, which affect up to 10% of the general population, represents a significant worldwide problem in the field of food safety in industrialized countries (Chafen et al., 2010). At the very top of the list of food allergens, shellfish is commonly identified as a cause of food hypersensitivity towards sensitized individuals. Ingestion of shellfish may result in immunoglobulin E (IgE)-mediated hypersensitivity provoking severe acute hypersensitivity reactions, which often involve the respiratory system, the gastrointestinal tract and the skin (Hajeb & Selamat, 2012; Sova et al., 2013). Recent data demonstrated seafood affect approximately 6.5 million people a year, among which about 0%–10.3% of the population suffers from shellfish allergy (Moonesinghe et al., 2014).

Until now, among thirteen different allergens identified in shellfish, tropomyosin (TM) is considered as the major allergen responsible for most shellfish-caused allergic reactions to most subject's IgE (Shafique, Inam, Ismail, & Chaudhary, 2012). TM is identified as a major heat-stable IgE-binding allergen in shellfish (Hoffman, Jr, & Miller, 1981; Shanti, Martin, Nagpal, Metcalfe, & Rao, 1993), which is a water-soluble myofibrillar protein with molecular weight as 35–38 kDa. Extensive researches show that the sequence of TM is highly conserved in different shellfish species, hence, the total serum IgE from almost 75%

of the individuals who are allergic to TM in one shellfish species can cross-react with that in other shellfish species (Tsabouri et al., 2012).

So far, correct and assertive food labelling is the only effective management for food allergy to help sensitized individuals strictly avoid the allergen-containing food. Currently, with the development of aquatic product processing technology, it becomes increasingly difficult to distinguish the present of seafood allergenic ingredients in some intensively processed food products, making sensitized individuals at higher risk contact with allergenic foods. Additionally, TM allergenicity may change during aquatic product processing. For example, cooking may deactivate TM allergenicity, while maillard modification may potentiate TM allergenicity (Nakamura, Watanabe, Ojima, Ahn, & Saeki, 2005). Therefore, it is urgently required to develop reliable, highly sensitive analytical methods for detecting low quantities of TM in complex food products in order to consummate shellfish allergens management.

Current analytical methods have been designed to trace TM in shellfish products. Enzyme-linked immunosorbent assay (ELISA) based on antigen-antibody reaction is considered as a commercially available methods to quantify shellfish TM in seafood raw materials (Zhang, Lu, Ushio, & Shiomi, 2014) and commercial shrimp, crab, and lobster extracts (Jeoung et al., 1997). Besides, real-time polymerase chain reaction (PCR) has been extensively applied to analyze TM in various

* Corresponding author.

** Corresponding author.

E-mail addresses: lzheng@hfut.edu.cn (L. Zheng), fulinglin@zjgsu.edu.cn (L. Fu).

shellfish extracts, such as Cal s 2 (*Callinectes sapidus*), Pen m 1 (*Penaeus monodon*), and Pen a 1 (*Penaeus aztecus*) (Eischeid, Kim, & Kasko, 2013). However, ELISA provides a significant rate of false positive results because of antibodies cross-reactivity, and is prone to false negative results due to denature or degrading epitopes during food processing (Gomaa & Boye, 2013). Moreover, real-time PCR could produce false positive results because it depends on the sequence of the target gene even if the related allergic proteins are not existed in samples.

Alternatively, biosensors are considered recently as a powerful tool for the detection and quantification of shellfish TM, offering high sensitivity and selectivity while allowing for site analysis, such as cell-based electrochemical biosensor (Donglei et al., 2013), Quartz crystal microbalance (QCM) biosensors (Sun et al., 2010). Surface plasmon resonance (SPR) based sensors, which show obvious advantages of high sensitivity, good selectivity, and high speed, are emerged as a real-time measurement for detecting multiple large and small molecules (Li, Lu, Zhang, Liu, & Qi, 2014). Currently, SPR phenomena has been widely utilized for development four types of sensing techniques, including the Kretschmann (And & Koji Suzuki, 2002), Otto (Tamm, Dawson, Connolly, Raza, & Gamble, 1991), fibre-optic types (Homola & Slavik, 2001) and localized surface plasmon resonance (LSPR) sensing (Willems & Van Duyne, 2007). On the basis of these sensing techniques, four types of interrogation: intensity, phase, wavelength and angle SPR based sensors has been successfully applied in the detection of some kinds of allergens. Pollet et al. (Pollet et al., 2011) presented a fiber optic SPR biosensor to detect peanut allergen Ara h1 with a detection limit of $0.1 \mu\text{g mL}^{-1}$. Ashley et al. (Ashley et al., 2018) developed a direct SPR technology for determination of cow's milk allergen β -lactoglobulin, giving a detection limit of $0.164 \mu\text{g mL}^{-1}$.

In the current work, we developed a novel direct immunoassay method for the major shellfish allergen TM detection using SPR biosensor with a gold patterned biochip covered by electrodeposited gold nanostructures to enhance the sensitivity of SPR biosensing. The major challenge in developing this detection systems for determining the TM is overcoming interference from shellfish complex sample matrixes. First, the monoclonal antibody against TM (mAb) is modified on the surface of gold patterned biochip. Besides, we evaluated SPR biosensor to detect TM in shellfish samples by determination of sensitivity, reproducibility and resolution.

2. Materials and methods

2.1. Materials and chemicals

Sodium carbonate, Sodium chloride, Citric acid trisodium salt, Potassium carbonate, Bull Serum Albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were of analytical grade. The shellfish TM and the anti-tropomyosin monoclonal antibody (TM-mAb) were purchased from Indoor Biotechnology (Charlottesville, VA, USA). DEAE-Sepharose fast flow was purchased from General Electric Company (Fairfield, Connecticut, USA). A total of 8 species of worldwide high-consumption of shellfish (*Litopenaeus vannamei*, *Fenneropenaeus chinensis*, *Penaeus monodon*, *Macrobrachium nipponense*, *Procambarus clarkia*, *Metapenaeus ensis*, *Exopalaemon carinicauda*, *Eriocheir sinensis*) were purchased from a local supermarket (CenturyMart, Hangzhou, China). All solutions were prepared using ultrapure water ($18.2 \text{ M}\Omega$) purified using a Milli-Q system (Millipore, Billerica, Massachusetts).

2.2. Equipment

The SPR measurement was carried out on the home-built angle modulation SPR biosensor. A polarized He-Ne laser centered at 650 nm is used as the excitation light source in *p* polarization. Light passes through a polarizer and then is reflected at a 50 nm layer of gold film coated on a glass slide, which was fixed on the surface of an angle

prism. The reflected light beam is then collimated by a cylindrical convex lens with a vertical axis of symmetry after which the light beam passes through a quarter-wave plate and another polarizer in turn. Another cylindrical convex lens with a horizontal axis of symmetry is used to image the pattern of the sample irradiated by the line-shaped beam on the gold film. Then a cylindrical concave lens with a vertical axis of symmetry is placed to expand the beam on the horizontal plane for enhancing the angular resolution of the system. At the end of the light path, there is a CCD camera (TCD1304DG). A personal computer controls the CCD to acquire digitized images with a dynamic range of 16 bits and a resolution of $8 \mu\text{m} \times 200 \mu\text{m}$ pixels.

2.3. Modification of sensor chip

The SPR measurements were performed on a thin gold layer (50 nm thickness) film deposited on a glass substrate produced by our research group. Bare gold sensor chips were chemical modified by cysteine solution, which was employed as a supporter to bind TM-mAb. Then, TM-mAb was injected into the reactor, followed by incubating for 1 h to immobilize TM-mAb on gold chips. After that, the chip was wash by deionized water to remove unbound TM-mAb. The sensor surface was blocked by injecting 5% (W/V) BSA solution, incubating for 1.5 h at 25°C to minimize nonspecific interaction of analytes.

2.4. Sample preparation

Shrimp muscle was ground into powder with liquid nitrogen, and 0.1 g of shrimp powder was mixed with 1 mL of mixture buffer (1 mL of Lysis Buffer, 1 μL of protease inhibitor, 10 μL of phosphodiesterases, 5 μL of 100 mM PMSF) at 4°C . Total shellfish protein extraction was performed using a commercial kit (KeyGEN, Nanjing, China), and protein extract was obtained after stationary incubation for 2 h at 4°C . Then, the extract was centrifuged in a Microfuge 22 R Centrifuge (Beckman Coulter, Mississauga, ON) at $10000 \times g$ at 4°C for 5 min and the supernatant was obtained. The total shellfish protein extraction was eluted with 20 mL of NaCl (0.5 M) at 1 mL min^{-1} in Tris-HCl buffer (pH 8.5) using 1 mL DEAE-Sepharose Fast Flow column by AKTApure 25 and the other nonallergenic proteins was collected. The concentration of total or the other nonallergenic proteins was determined by bicinchoninic acid (BCA) assay (Pierce, Rockford, USA) with bovine serum albumin (BSA) as the standard. The purity of TM in shellfish extracts and the other nonallergenic proteins was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using AlphaView SA 3.4.0 software.

2.5. SPR assay protocol

A standard of TM ($2.04 \mu\text{g mL}^{-1}$) was prepared in 0.01 M Tris-HCl Buffer. From this, the TM solution was consecutively diluted to 3.12, 6.25, 12.50, 25.00, $50.00 \mu\text{g mL}^{-1}$ as the working solutions. 200 μL of each sample, includes working solutions and sample solutions, were respective added to gold chips with flow rate being $20 \mu\text{L min}^{-1}$ at 37°C . After incubation, SPR signals were collected for each sample. After each sample injection, 100 μL of 0.1 M NaOH was implemented with flow rate at $400 \mu\text{L min}^{-1}$ to regenerate the surface of mAb modified gold chip and 10 mM PBS (pH 7.2) was used as the running buffer. All the data were processed using Origin Lab 2018 software.

3. Results and discussion

3.1. Sensor surface functionalization

This SPR biosensor was designed based on Kretschmann configuration, in which a chip with a flat metal layer on a transparent substrate is placed on a right-angled prism. At the interface between the metal later and the substrate, incident light is totally reflected, exciting

surface plasmon waves on the metal surface only at a specific incident angle (the SPR angle) (And & Koji Suzuki, 2002). The sensitivity of Kretschmann-type SPR sensors depends quite a lot on the type and thickness of the metal film in the SPR module (Thammacharoen, Yu, Ekgasit, & Knoll, 2005). For allergenic protein detection assays, gold nanoparticles (AuNPs) are the most studied nanostructured materials to construct SPR affinity sensor chips to amplify the signal (angle shift) and improve sensitivity due to the highly stable optical and chemical properties of AuNPs (T A, C A, & R L, 2000; Xu et al., 2015). The shifts in surface plasmon resonance wavelengths are amplified by a factor of 3 because of the resonance coupling between a molecular absorption band in the analytes and the nanoparticle plasmon band (Zhao, Das, Schatz, Sligar, & Duyne, 2008). In addition, consistent with the studies that gold layer made from 50 nm AuNPs present the stronger protein binding signal, a flat gold layer (50 nm) formed by physical vapour deposition on a transparent substrate is placed on a prism to construct the SPR affinity sensor chip (Xu et al., 2013).

As the aim was to establish a method for determination of TM in shellfish samples using SPR biosensor, we investigated two influence factors for immobilizing anti-TM mAb on the surface of SPR gold chip, including the anti-TM mAb concentration, and block buffer concentration. The mAb concentration is a significant factor to affect the sensitivity. As shown in Fig. 1A, among three concentrations of TM-mAb, the maximum response shift was observed while $1.3 \mu\text{g mL}^{-1}$ TM-mAb was injected into the reactor. A monolayer of TM-mAbs was absorbed on the surface of gold biochip by physical adsorption through electrostatic force, depending on the orientation of immobilisation. Hence, the optimal TM-mAb concentration of $1.3 \mu\text{g mL}^{-1}$ was selected. After immobilisation, in order to block the non-specific site on AuNPs and improve the specificity of this method, the optimal BSA concentration was investigated. As shown in Figs. 1B and 5% (W/V) BSA solution was the optimal block buffer at which the smallest signal shift (close to zero) was obtained. Theoretically, BSA covalently bonded to AuNPs through one or more of its 35 cysteine residues (Siriwardana, Wang, Vangala, Fitzkee, & Zhang, 2013).

3.2. TM immunoassay strategies on the SPR

In this method, the presence of TM in shellfish samples was detect for binding to the TM-mAb modified gold chip in a direct manner. In order to develop the method using SPR biosensor with gold patterned biochips with good sensitivity, accuracy and stability, which can

rapidly analyze the major allergen TM of shellfish, three influence factors were taken into consideration, including the pH of the running buffer, flow rate of injection of the sample, and sample volume. First, we investigated the impact of the pH of the running buffer on the signal intensity of (Fig. 2A). The pH value of the running buffer can affect the combination of antigen-antibody, further affecting sensitivity and accuracy. According to hypothesis that the antigen-antibody complexes are stable between pH 5 and 9 (Steiner, 1955), three pH (5.0, 7.2, 9.0) of the running buffer were tested. As shown in Fig. 2A, SPR signal was the strongest at pH 7.2 of 10 mM PBS running buffer. Hence, an optimum pH of 10 mM PBS running buffer was selected. Second, the flow rate of sample injection with 10 mM PBS (pH 7.2) running buffer were examined (Fig. 2B). Higher flow rate of sample injection can effectively shorten the detection time, however, the excessive flow rate may reduce the reaction time between antigen antibody, which can influence the signal intensity. The results showed that the relatively strong degree of signal intensity was obtained at $10 \mu\text{L min}^{-1}$ and $20 \mu\text{L min}^{-1}$. On the basis of the results of this study, an optimum flow rate of sample injection of $20 \mu\text{L min}^{-1}$ was selected, showing optimal signal intensity with a shorter detection time. Finally, we demonstrated the impact of sample volume on the signal intensity of TM with 10 mM PBS (pH 7.2) running buffer at $20 \mu\text{L min}^{-1}$ (Fig. 2C), indicating that the similar signal shift was achieved at 200 μL and 300 μL of sample volume. Collectively, we decided to employ a pH of 10 mM PBS running buffer and flow rate of sample injection of $20 \mu\text{L min}^{-1}$ as well as sample volume of 200 μL as the optimal parameters for TM detection by SPR biosensor.

3.3. Method validation

On the basis of the above optimal conditions, this method was validated in terms of linear range, limit of detection (LOD), limit of quantification (LOQ), specificity, precision (relative standard deviation, RSD) and accuracy (recovery).

3.3.1. Linearity, LOD and LOQ

Depending on these optimal assay conditions, this method has been validated in terms of linear range, limit of detection (LOD) and limit of quantification (LOQ) using standards of TM. Five different concentrations of TM standards (3.12, 6.25, 12.50, 25.00, 50.00 $\mu\text{g mL}^{-1}$) were injected in series to determine the linear range of this method. As show in Fig. 3, the signal shift increases with increasing the concentrations of

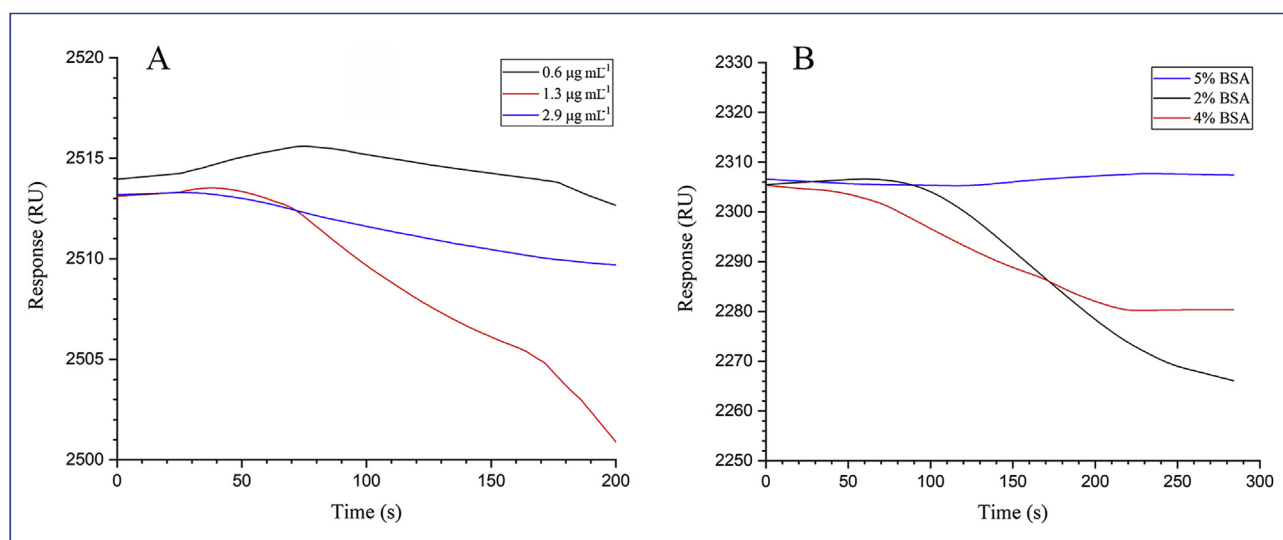


Fig. 1. SPR sensorgram showing the effects of anti-TM mAb concentration and BSA concentration on the SPR response shift of tropomyosin standards. (A) The SPR curves of different concentrations of anti-TM mAb; (B) The SPR curves of different concentrations of BSA.

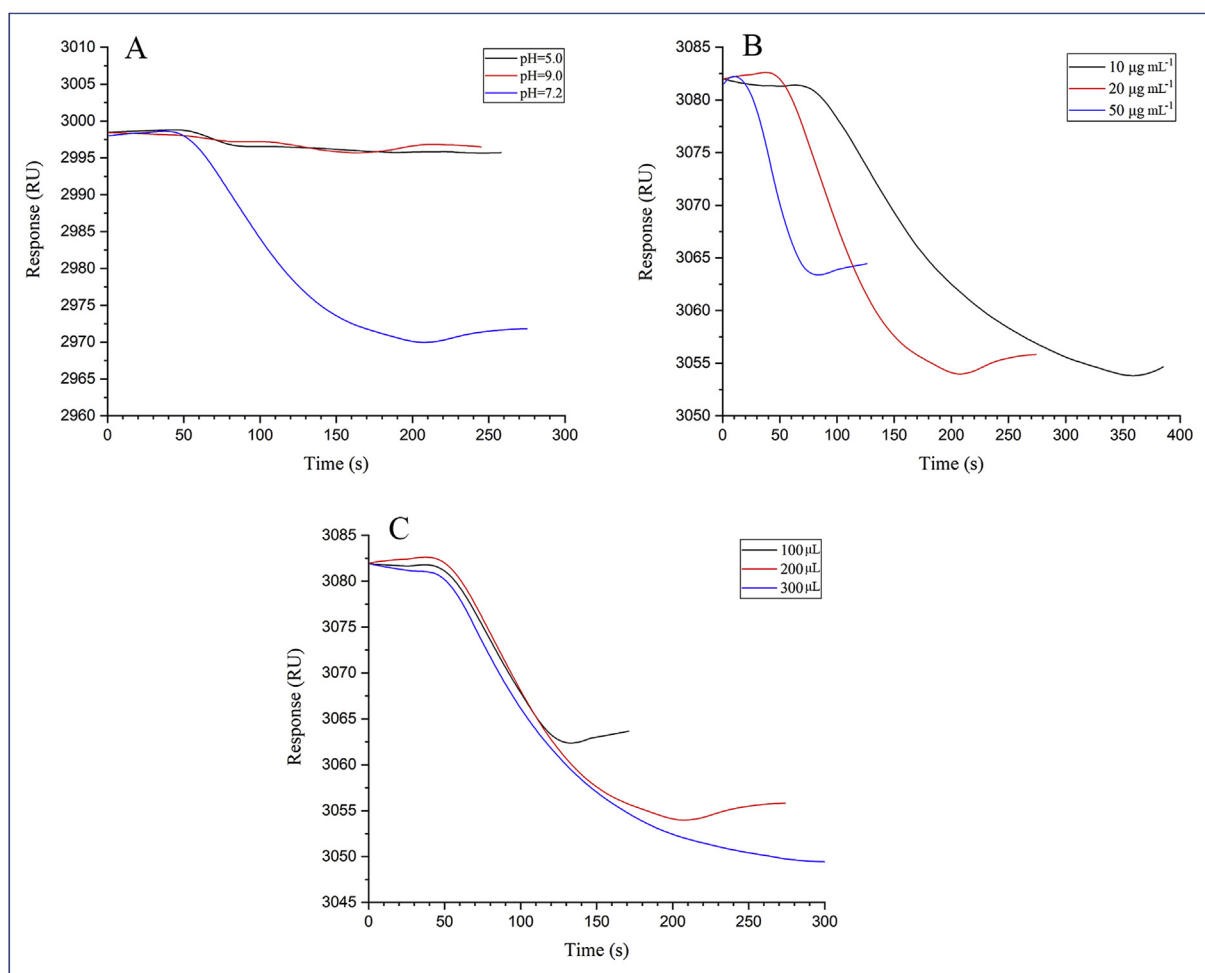


Fig. 2. SPR sensorgram for TM standards. (A) 200 μL sample volume with flow rate of sample injection at $20 \mu\text{L min}^{-1}$ under pH values of running buffer. (B) 10 mM PBS (pH 7.2) with 200 μL sample volume under different flow rates of sample injection. (C) 10 mM PBS (pH 7.2) with flow rate of sample injection at $20 \mu\text{L min}^{-1}$ under different sample volumes.

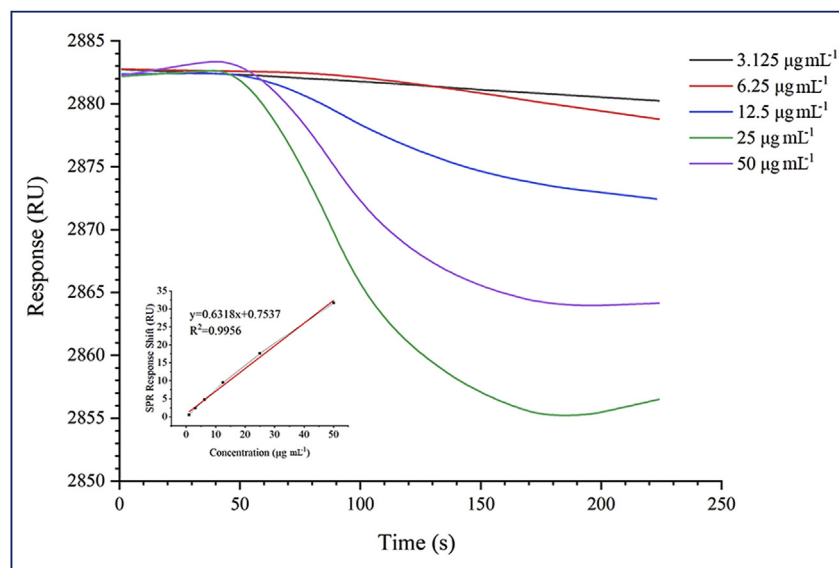


Fig. 3. SPR sensorgram of tropomyosin standards at different concentrations and standard curve. Experiment conditions: 10 mM PBS (pH 7.2), 200 μL sample volume with flow rate of sample injection at $20 \mu\text{L min}^{-1}$.

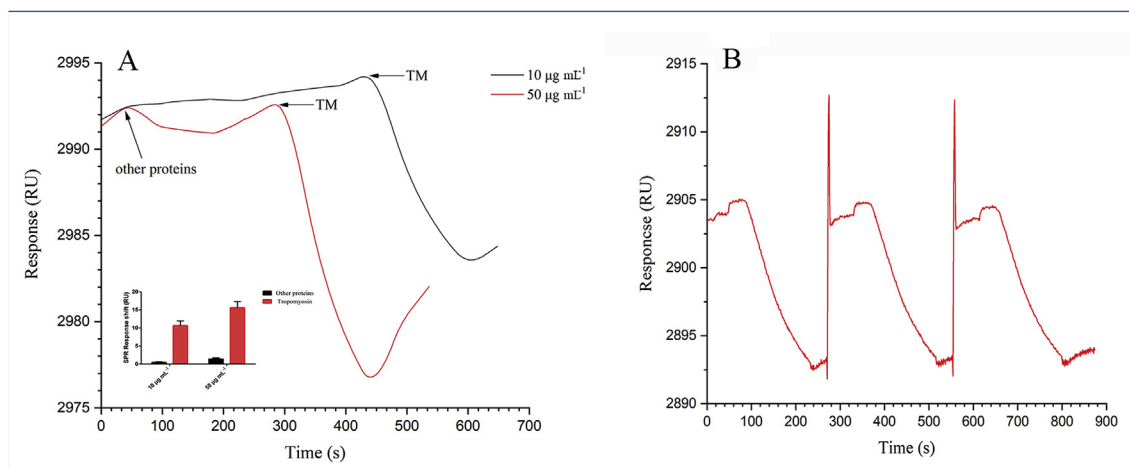


Fig. 4. SPR sensorgram of the other nonallergenic proteins in shellfish and tropomyosin standards. (A) Relative responses of the other nonallergenic proteins in shellfish towards the sensor surface; (B) SPR sensorgram of tropomyosin standards under continuous injection. Experiment conditions: 10 mM PBS (pH 7.2), 200 µL sample volume with flow rate of sample injection at 20 µL min⁻¹.

TM standards. Hence, there is a linear relationship between the signal shift and the concentration of TM standards ranged from 3 to 50 µg mL⁻¹, which could be represented by the equation: $y = 0.6318x + 0.7537$ with good correlation ($R^2 = 0.9956$). We also determined that the limit of detection (LOD) as 1.0 µg mL⁻¹ ($S/N = 3$), and the limit of quantitation (LOQ) was 2.5 µg mL⁻¹ ($S/N = 10$). Compared to ELISA which reported a LOD of 0.09 µg mL⁻¹ (Zhang et al., 2014), the obtained LOD of this method reported here was higher. However, this method is more suitable for determination of TM in actual specimens since the concentrations of TM are relatively high in shellfish. Generally, according to the Japanese Food Allergen Labeling Regulation, the threshold of food allergen is 10 µg of allergen protein/g (or mL) of food taken by individuals (Shoji, Adachi, & Akiyama, 2017). Besides, another study indicated that ingestion of 2.5 g of shrimp protein could cause objective eliciting reactions in 10% of 436 allergic patients during oral food challenge (Ballmer-Weber et al., 2015).

3.3.2. Specificity, precision and accuracy

Additionally, the other nonallergenic proteins (the total protein in the shrimp except for TM) in shellfish samples were treated to investigate specificity, precision (intra and inter-day relative standard deviation, RSD) and accuracy (recovery) of this method. In order to explore the specificity of this method to detect TM in shellfish, the blank samples (other nonallergenic proteins) in shellfish were tested (Fig. 4A). We firstly injected two concentrations of blank samples, and then, the same concentrations of TM standards were injected to make sure the normal function of this method. Fig. 4A showed that the SPR response were close to zero when the blank samples at concentrations of 5 and 10 µg mL⁻¹ were injected, while the signal shift were 10–15 with the injection of TM standards, indicating that the non-specific binding of shellfish samples on the surface of gold chip can be ignored.

Moreover, we investigated the intra-day and inter-day RSD for the signal shift by continuously injecting three different concentrations (5, 10, 20 µg mL⁻¹) of TM standards for three times (Fig. 4B), respectively. The results summarized in Table S1 demonstrated that intra-day RSD was 0.6–4.5%, while inter-day RSD was 1.7–6.8%, suggesting that this method we established is reliable.

Finally, the recovery test was performed by blank samples mixed with TM standards at concentrations of 5, 10, 20 µg mL⁻¹, respectively. The recovery of this method ranged from 92.0% to 108.0% (Fig. S2 and Table 1), indicating the good accuracy of this method.

Table 1

Percentage of recovery (n = 3) for determination of tropomyosin in shellfish samples.

Sample No.	Background values (µg mL ⁻¹)	Actual quantity (µg mL ⁻¹)	Measured value (µg mL ⁻¹) ± SD ^a	Recovery (%)
S1	0	5	4.7 ± 0.06	95
	0	10	9.6 ± 0.16	96
	0	20	18.4 ± 0.11	92
S2	0	5	5.4 ± 0.05	108
	0	10	10.3 ± 0.13	103
	0	20	18.6 ± 0.15	93
S3	0	5	5.1 ± 0.02	101
	0	10	9.7 ± 0.12	97
	0	20	20.4 ± 0.15	102

^a n = 3.

3.4. Analysis of shellfish samples

In order to test the ability of this method to function effectively and applicably in practical applications to trace the presence of TM in shellfish samples, we analyzed eight different species of fresh shellfish samples from a local market and performed a quick procedure to obtain the total protein of these shellfish. Fig. S1 described the SDS-PAGE electropherogram of the total protein extracted from eight different shellfish. It is observed the bands (35–38 kDa) of TM in all the shellfish samples, indicating that the major shellfish allergen TM extensively existed in different shellfish species. The concentrations of TM in shellfish samples were back-calculated from the calibration curves. The results are summarized in Table 2 and the proportions of TM in the total protein of shellfish samples were shown in Fig. S3. These data showed that TM could be detected in all of the shellfish species we collected.

Table 2

Amounts of TM in shellfish samples detected by SPR biosensor.

Shellfish species	Measured Conc. (mg g ⁻¹) ± SD ^a
<i>Litopenaeus vannamei</i>	0.80 ± 0.004
<i>Fenneropenaeus chinensis</i>	0.83 ± 0.002
<i>Penaeus monodon</i>	1.82 ± 0.005
<i>Macrobrachium nipponense</i>	0.64 ± 0.005
<i>Procambarus clarkii</i>	1.11 ± 0.056
<i>Metapenaeus ensis</i>	0.49 ± 0.031
<i>Exopalaemon carinicauda</i>	0.89 ± 0.003
<i>Eriocheir sinensis</i>	0.54 ± 0.001

with abundances between 0.49 and 1.82 mg g⁻¹. Interestingly, the content of TM in fresh *Litopenaeus vannamei* and *Penaeus monodon* obtained by ELISA (Lin, Lee, Huang, Sheu, & Yu, 2018) were higher than we tested here, while the content of TM in cooked shellfish were close, which may due to the discrepancy between two methods. Our analysis of fresh samples provides information about the amounts of TM in eight main shellfish species that are frequently consumed and highlights the possibility of the newly developed SPR-based method for detecting the major allergen TM in shellfish, which may help the industrial and government allergen-labeling of shellfish and shellfish products.

4. Conclusion

In summary, this paper demonstrated a novel Kretschmann-type SPR sensor with a gold chip for rapid and accurate detection of shellfish allergen TM, with good sensitivity, specificity and stability. In addition, with good accuracy and precision, this method can overcome the problem of antibody cross-reactivity with the other nonallergenic proteins in shellfish samples, showing the excellent specificity. Moreover, this method can accurately quantify TM from 8 different species with simple sample preparation. Further work is underway to highlight the applicability of this method for the rapid and accurate detection of diverse allergens in shellfish products at every stage of productive process in the future.

Conflicts of interest

All Authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with animals performed by any of the authors.

Informed consent

Not applicable.

Acknowledgments

This work was financially supported by the Zhejiang Provincial Natural Science Foundation for Distinguished Young Scholars of China (Grant number: LR19C200001).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodcont.2019.02.041>.

References

- And, K. K., & Suzuki, K. (2002). Theoretical understanding of an absorption-based surface plasmon resonance sensor based on Kretschmann's theory. *Analytical Chemistry*, 74(3), 696–701.
- Ashley, J., D'Aurelio, R., Piekarska, M., Temblay, J., Pleasants, M., Trinh, L., et al. (2018). Development of a β -lactoglobulin sensor based on SPR for milk allergens detection. *Biosensors*, 8(2), 32–42.
- Ballmer-Weber, B. K., Montserrat, F. R., Kirsten, B., Marianne, D., Matthew, S., Mackie, A. R., et al. (2015). How much is too much? Threshold dose distributions for 5 food allergens. *The Journal of Allergy and Clinical Immunology*, 135(4), 964–971.
- Chafen, J. J., Newberry, S. J., Riedl, M. A., Bravata, D. M., Maglione, M., Suttrop, M. J., et al. (2010). Diagnosing and managing common food allergies: A systematic review. *Jama*, 303(18), 1848–1856.
- Donglei, J., Jian, J., Lu, A., Xiulan, S., Yinzhi, Z., Genyi, Z., et al. (2013). Mast cell-based electrochemical biosensor for quantification of the major shrimp allergen Pen a 1 (tropomyosin). *Biosensors and Bioelectronics*, 50(24), 150–156.
- Eischeid, A. C., Kim, B. H., & Kasko, S. M. (2013). Two quantitative real-time PCR assays for the detection of penaeid shrimp and blue crab, crustacean shellfish allergens. *Journal of Agricultural and Food Chemistry*, 61(24), 5669–5674.
- Gomaa, A., & Boye, J. I. (2013). Impact of thermal processing time and cookie size on the detection of casein, egg, gluten and soy allergens in food. *Food Research International*, 52(1), 483–489.
- Hajeb, P., & Selamat, J. (2012). A contemporary review of seafood allergy. *Clinical Reviews in Allergy and Immunology*, 42(3), 365–385.
- Hoffman, D. R., Day, E. D., & Miller, J. S. (1981). The major heat stable allergen of shrimp. *Annals of Allergy*, 47(1), 17–22.
- Jeoung, B. J., Reese, G., Hauck, P., Oliver, J. B., Daul, C. B., & Lehrer, S. B. (1997). Quantification of the major brown shrimp allergen Pen a 1 (tropomyosin) by a monoclonal antibody-based sandwich ELISA. *The Journal of Allergy and Clinical Immunology*, 100(2), 229–234.
- Li, J., Lu, D. F., Zhang, Z., Liu, Q., & Qi, Z. M. (2014). Hierarchical mesoporous silica film modified near infrared SPR sensor with high sensitivities to small and large molecules. *Sensors and Actuators B: Chemical*, 203(21), 690–696.
- Lin, S. Y., Lee, C. H., Huang, E. S., Sheu, S. C., & Yu, H. S. (2018). Quantification of Crustacean tropomyosin, a major food allergen, in eight species of Taiwanese shrimp based on immunoassay. *Food Analytical Methods*, 11(9), 2607–2613.
- Moonesinghe, H. R., Kilburn, S., Mackenzie, H., Turner, P. J., Venter, C., Lee, K., et al. (2014). Prevalence of fish and shellfish allergy: a systematic review. *The Journal of Allergy and Clinical Immunology*, 133(2), AB202.
- Nakamura, A., Watanabe, K., Ojima, T., Ahn, D. H., & Saeki, H. (2005). Effect of maillard reaction on allergenicity of scallop tropomyosin. *Journal of Agricultural and Food Chemistry*, 53(19), 7559–7564.
- Pollet, J., Delpont, F., Janssen, K. P., Tran, D. T., Wouters, J., Verbiest, T., et al. (2011). Fast and accurate peanut allergen detection with nanobead enhanced optical fiber SPR biosensor. *Talanta*, 83(5), 1436–1441.
- Shafique, R. H., Inam, M., Ismail, M., & Chaudhary, F. R. (2012). Group 10 allergens (tropomyosins) from house-dust mites may cause covariation of sensitization to allergens from other invertebrates. *Allergy & Rhinology*, 3(2), e74.
- Shanti, K. N., Martin, B. M., Nagpal, S., Metcalfe, D. D., & Rao, P. V. (1993). Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. *The Journal of Immunology*, 151(10), 5354–5363.
- Shoji, M., Adachi, R., & Akiyama, H. (2017). Japanese food allergen labeling regulation: An update. *Journal of AOAC International*, 101(1), 8–13.
- Siriwardana, K., Wang, A., Vangala, K., Fitzkee, N., & Zhang, D. (2013). Probing the effects of cysteine residues on protein adsorption onto gold nanoparticles using wild-type and mutated GB3 proteins. *Langmuir the ACS Journal of Surfaces & Colloids*, 29(35), 10990–10996.
- Slavik, R., Homola, J., Čtyroký, J., & Brynda, E. (2001). Fibre-optic sensor based on surface plasmon resonance. *Sensors and Actuators B: Chemical*, 74(1–3), 106–111.
- Sova, C., Feuling, M. B., Bauml, M., Gleason, L., Tam, J. S., Zafra, H., et al. (2013). Systematic review of nutrient intake and growth in children with multiple IgE-mediated food allergies. *Nutr. Clin. Pract.* 28(6), 669–675 Official Publication of the American Society for Parenteral & Enteral Nutrition.
- Steiner, R. F. (1955). Reversible association processes of globular proteins. IX. The effect of pH and electrolytes upon an antigen-antibody combination. *Archives of Biochemistry and Biophysics*, 55(1), 235–252.
- Sun, X., Zhang, Y., Shao, J., Shen, L., Qian, H., & Zhu, W. (2010). A quartz crystal microbalance-based immunosensor for shrimp allergen determination in food. *European Food Research and Technology*, 231(4), 563–570.
- T A, T., C A, M., & R L, L. (2000). Scanometric DNA array detection with nanoparticle probes. *Science*, 289(5485), 1757–1760.
- Tamm, I. R., Dawson, P., Connolly, M. P., Raza, S. H., & Gamble, H. S. (1991). Characterization of surface plasmons on metal-oxide-semiconductor structures. *Optica Acta International Journal of Optics*, 38(8), 1593–1598.
- Thammacharoen, C., Yu, F., Ekgasit, S., & Knoll, W. (2005). Influence of the metal film thickness on the sensitivity of surface plasmon resonance biosensors. *Applied Spectroscopy*, 59(5), 661–667.
- Tsabori, S., Triga, M., Makris, M., Kalogeromitos, D., Church, M. K., & Priftis, K. N. (2012). Fish and shellfish allergy in children: Review of a persistent food allergy. *Pediatric Allergy & Immunology*, 23(7), 608–615.
- Willems, K. A., & Van Duyn, R. P. (2007). Localized surface plasmon resonance spectroscopy and sensing. *Annual Review of Physical Chemistry*, 58(1), 267–297.
- Xu, L., Ma, W., Wang, L., Xu, C., Kuang, H., & Kotov, N. A. (2013). Nanoparticle assemblies: Dimensional transformation of nanomaterials and scalability. *Chemical Society Reviews*, 42(7), 3114–3126.
- Xu, L., Yan, W., Ma, W., Kuang, H., Wu, X., Liu, L., et al. (2015). SERS encoded silver pyramids for attomolar detection of multiplexed disease biomarkers. *Advanced Materials*, 27(10), 1706–1711.
- Zhang, H., Lu, Y., Ushio, H., & Shiomi, K. (2014). Development of sandwich ELISA for detection and quantification of invertebrate major allergen tropomyosin by a monoclonal antibody. *Food Chemistry*, 150, 151–157.
- Zhao, J., Das, A., Schatz, G. C., Sligar, S. G., & Duyn, R. P. V. (2008). Resonance localized surface plasmon spectroscopy: Sensing substrate and inhibitor binding to cytochrome P450. *Journal of Physical Chemistry C*, 112(34), 13084–13088.

Quantification of Shellfish Major Allergen Tropomyosin by SPR Biosensor with Gold Patterned Biochips

Jinru Zhou^{1,2}, Yanbo Wang², Yifan Qian², Teng Zhang³, Lei Zheng^{1,*}, Linglin Fu^{2,‡}

¹School of Food Science and Engineering, Hefei University of Technology, Hefei, 230009, China

²Zhejiang Engineering Institute of Food Quality and Safety, Zhejiang Gongshang University, Hangzhou 310018, China

³ZhongLongYiCheng Technology company, Beijing 100166, China

Supporting information

Fig. S1. SDS-PAGE electrophoretograms of shrimp samples from 8 different shrimps. M, molecular weight markers; 1, *Litopenaeus vannamei*; 2, *Fenneropenaeus chinensis*; 3, *Penaeus monodon*; 4, *Macrobrachium nipponense*; 5, *Procambarus clarkia*; 6, *Metapenaeus ensis*; 7, *Exopalaemon carinicauda*; 8, *Eriocheir sinensis*.

Fig. S2. SPR sensorgram of tropomyosin detection in blank samples at different concentrations. Experiment conditions: 10 mM PBS (pH 7.2), 200 μ L sample volume with flow rate of sample injection at 20 μ L min⁻¹.

Fig. S3. SPR sensorgram of TM analysis for shrimp sample. Experiment conditions: 10 mM PBS (pH 7.2), 200 μ L sample volume with flow rate of sample injection at 20 μ L min⁻¹.

Table S1. Intra-day and inter-day RSD (n = 3) for determination of tropomyosin.

Fig. S1

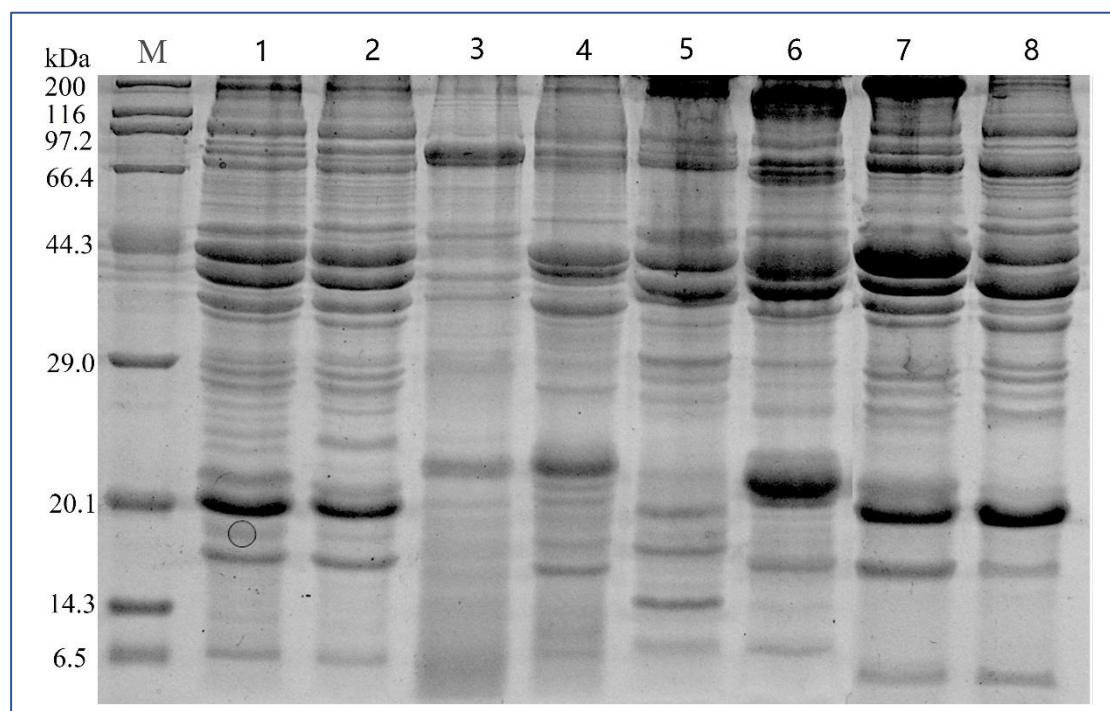


Fig. S2

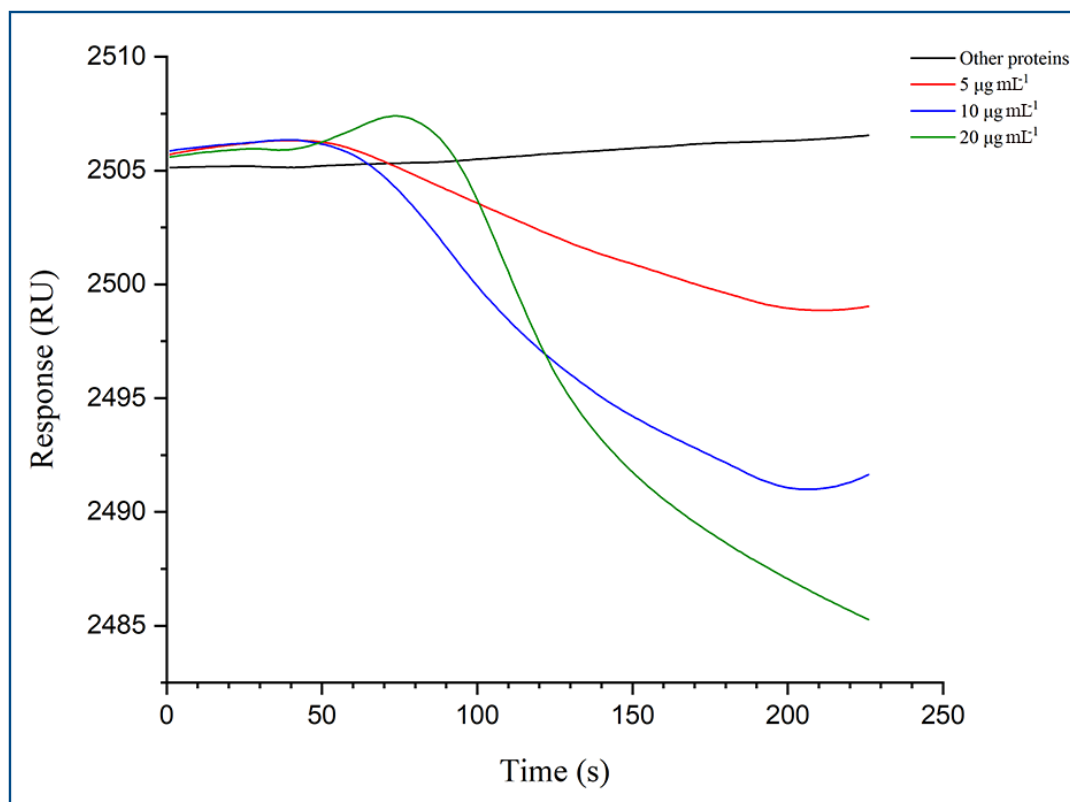


Fig. S3

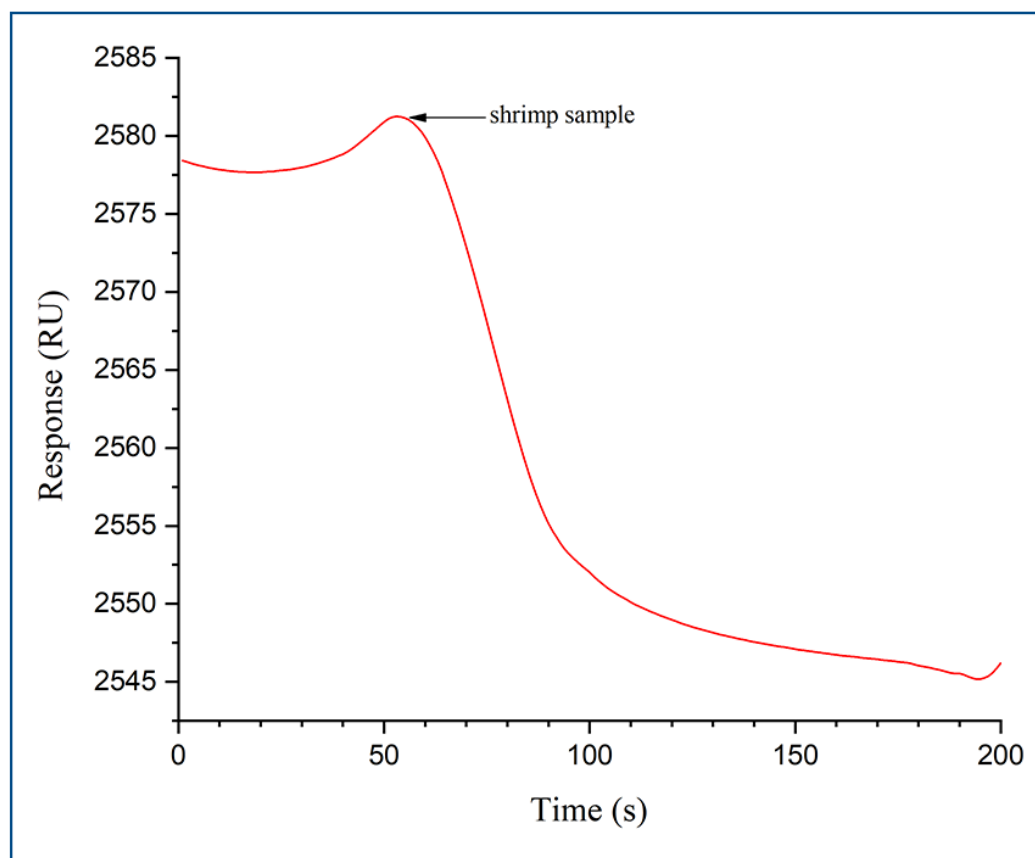


Table S1 Intra-day and inter-day RSD (n = 3) for determination of tropomyosin.

Conc. ($\mu\text{g mL}^{-1}$)	Inter-day RSD (n=3)	Intra-day RSD (n=3)
5	1.7	0.6
10	5.4	2.3
20	6.8	4.5