Aptameric biosensor for the sensitive detection of major shrimp allergen, tropomyosin

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Abstract

The development of a sensitive and rapid detection approach for allergens in various food matrices is essential to assist patients in managing their allergies. The most common methods used for allergen detection are based on immunoassays, PCR and mass spectrometry. However, all of them are very complex and time-consuming. Herein, an aptamer biosensor for the detection of the major shrimp allergen tropomyosin (TM) was developed. Graphene oxide (GO) was used as a platform for screening of the minimal-length aptamer sequence required for high-affinity target binding. A fluorescein dye labeled GO quenches the truncated aptamer by π-stacking interactions. After the addition of TM, the fluorescence was restored due to the competitive binding of the aptamer to GO. One of the truncated aptamers was found to bind to TM with four-fold higher affinity (30 nM) compared to the full-length aptamer (124 nM), with a limit of detection (LOD) of 2 nM. The aptamer-based sensor demonstrates the sensitive, selective, and specific detection of TM in 30 min. The performance of the sensor was confirmed using TM spiked chicken soup, resulting in a high percentage recovery (~97±10%). The association of GO and labelled aptamer sensor platform has shown the rapid detection of TM in food, which is compared to other methods very sensitive, specific and performs in high throughput application.

Keywords: Aptasensor, analytical assay, graphene oxide, tropomyosin, fluorescence assay, shellfish-allergens, allergen detection, food safety.

1. Introduction

The prevalence of shellfish allergy in the increasing worldwide affecting up to 5% of consumers. Shellfish are divided into two groups, the crustaceans and mollusks, including commonly consumed species including shrimps, crabs, lobsters and gastropods, cephalopods and bivalves respectively. Shrimp is the predominant species causing over 80% of reactions to shellfish that result in severe clinical outcomes. Tropomyosin is one of the major allergic proteins found in shrimp and highly stable even at high temperatures(Lopata, Kleine-Tebbe, & Kamath, 2016).

The current allergen detection methods are mainly based on enzyme-linked immunosorbent assay (ELISA), DNA detection, microarray(Lupinek, Wollmann, Baar, Banerjee, Breiteneder, Broecker, et al., 2014) and qualitative/semi-quantitative lateral flow assays (Sharma, Khuda, Parker, Eischeid, & Pereira, 2016). ELISA is the most commonly used method for the TM detection and quantification (Fuller, Goodwin, & Morris, 2006; Kamath, Abdel Rahman, Komoda, & Lopata, 2013; Kamath, Thomassen, Saptarshi, Nguyen, Aasmoe, Bang, et al., 2014; Seiki, Oda, Yoshioka, Sakai, Urisu, Akiyama, et al., 2007; Werner, Fæste, & Egaas, 2007; Y. Zhang, Wu, Wei, Zhang, & Mo, 2017). The homology in amino acid (AA) sequence of TM between crustaceans and mollusks ranges from 55-65% (Ruethers, Taki, Johnston, Nugraha, Le, Kalic, et al., 2018). However, among the crustacean group the sequence homology ranges from 88-100%, with the highest homology between different shrimp species with >95% homology. Due to this various levels of aa sequence homology, the resulting potential immunological cross-reactivities pose a challenge for antibody based ELISAs to distinguish between crustaceans and mollusks TM (Fernandes, Costa, Oliveira, & Mafra, 2015; Kamath, Abdel Rahman, Komoda, & Lopata, 2013).

Mass spectrometry has been applied for the detection and quantification of food allergens to overcome the drawbacks of immunological-based techniques. Allergens from several shellfish species prawns (Khanaruksombat, Srisomsap, Chokchaichamnankit, Punyarit, & Phiriyangkul, 2014; Koeberl, Clarke, & Lopata, 2014; Koeberl, Kamath, Saptarshi, Smout, Rolland, O'Hehir, et al., 2014), crab (Koeberl, Clarke, & Lopata, 2014) and cephalopod (Shen, Cao, Cai, Ruan, Mao, Su, et al., 2012) have been analyzed utilizing different mass spectrometric techniques. Abdel Rahman et. al. have identified signature peptides for Black Tiger prawn, Northern prawn, and Snow crab, and then utilized these peptides for developing liquid chromatography (LC) coupled with multiple reaction monitoring (MRM) mass spectrometer methods for species-specific allergens quantification(Abdel Rahman, Kamath, Gagné, Lopata, & Helleur, 2013; Rahman, Gagné, & Helleur, 2012; Rahman, Kamath, Lopata, & Helleur, 2010; Rahman, Kamath, Lopata, Robinson, & Helleur, 2011; Rahman, Lopata, Randell, & Helleur, 2010). However, mass spectrometric analysis, immunoassays and PCR techniques are time-consuming and rely on skilful operators and expensive instruments. Therefore, rapid and low-cost biosensors, which can be used as point-of-care for the detection of TM is highly needed to help allergic patients with their preventive measures. Several biosensor-based techniques have been developed for the sensitive detection of TM from food sources. A recent report on the ultrasensitive detection of TM using aptamer-based photoelectrochemical methods and utilizing graphitic carbon nitride-TiO_2 nanocomposite (Amouzadeh Tabrizi, Shamsipur, Saber, Sarkar, & Ebrahimi, 2017) overcomes the disadvantages of the antibody-based allergen detection.

Aptamers consist of single-stranded DNA, RNA or peptides that bind to the target molecule with high affinity (K_{ds} in nanomolar to the picomolar range) and specificity. Aptamers are selected for a vast variety of targets including metal ions, pathogens, small organic

molecules, and viruses. Systematic Evolution of Ligands by Exponential Enrichment (SELEX) method is used to deterime the right aptamer for the targeted analyte from a library of chemically synthesised random sequences (containing approximately 10^{15} oligonucleotides). Biosensors developed for the detection of targets using the right aptamer as a recognition receptor. Aptamer-based biosensors have shown multiple advantages over antibodies such as in vitro selection, high stability, low cost of chemical synthesis and post modifications(Lapa, Chudinov, & Timofeev, 2016). The ideal aptamer consists of 40-100 nucleotides. Under suitable conditions, the aptamer can form secondary and tertiary structured binding pockets which can capture the target to form strong aptamer-target complexes.

Aptamer-based sensors undergo a significant conformational change upon target recognition, which leads to a change in the transducer signals. Though the aptamers are specific to their targets, post SELEX optimizations such as eliminating non-essential nucleotide will improve the affinity and the specificity considerably. A significant improvement in the binding affinity of anti-Salmonella enteritis and anti-progesterone truncated aptamer have been achieved by removing the non-essential nucleotides from the parental aptamer (H. Alhadrami, R. Chinnappan, S. Eissa, A. A. Rahamn, & M. Zourob, 2017; Chinnappan, AlAmer, Eissa, Rahamn, Salah, & Zourob, 2018). Zhang et al. have reported 40 nucleotide ssDNA aptamer obtained from the dot-blotting SELEX (Y. Zhang, Wu, Wei, Zhang, & Mo, 2017). As GO is an efficient energy acceptor (Ding, Cargill, Das, Medintz, & Claussen, 2015; Yang, Asiri, Tang, Du, & Lin, 2013), we used GO as a sensing platform for probing the high-affinity binding sequences from wild-type aptamer of anti-TM.

This study aims to develop an aptamer-based biosensor for the sensitive detection of the major shellfish allergen TM, using high-affinity fluorescein truncated ssDNA aptamers and GO as a sensing platform. Anti-TM aptamers has been reported recently, however the cross-

reactivity of the sensor was neither examined or validated with close relevant proteins or real samples(Y. Zhang, Wu, Wei, Zhang, & Mo, 2017). In this study we probed for high-affinity binding sequences from the parental aptamer to develop an efficient aptasensor. Cross-reactivity studies with different TM proteins derived from Shrimp, Oyster and anisakis were evaluated and further validation studies of the sensor were conducted to analyze and estimated the amount of spiked protein in the chicken noodle soup. We demonstrate here that the new developed sensor has high sensitivity for TM from shrimp and can be used for the analysis of food for contamination.

2. Experimental

2.1 Materials and Methods

Natural tropomyosin from black tiger prawn (*Penaeus monodon*) and Oyster (*Crassostrea gigas*) were purified using ammonium sulphate precipitation method as described previously (Nugraha, Kamath, Johnston, Zenger, Rolland, O'Hehir, et al., 2018). Recombinant prawn (*Penaeus monodon*) tropomyosin (rPen m 1) and recombinant anisakis (*Anisakis simplex*) tropomyosin (rAnis3) was expressed and purified as described previously (Asnoussi, Aibinu, Gasser, Lopata, & Smooker, 2017; Koeberl, et al., 2014). N-Hydroxysuccinimide (NHS) activated sepharose-4B was purchased from GE healthcare (Milwakee, WI, USA). Phosphate buffered saline (PBS) tablets, Tris base, sodium chloride, sodium acetate, sodium bicarbonate, sodium azide hydrochloric acid, magnesium chloride streptavidin and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St Louis, MO, USA). Graphene oxide (GO) dispersion was supplied by Dropsens Inc. (Asturias, Spain). HPLC purified labeled, and unlabeled oligonucleotides were provided by Metabion International (Planegg, Germany). The DNA oligonucleotides were dissolved in ultrapure Milli-Q water to make the stock solutions and stored at -20°C until further use. The DNA solutions used in the

experiments were diluted with binding buffer. The fluorescein-labeled oligonucleotides were protected from light while performing the experiments.

2.2 Fluorescence Measurements

All the fluorescence measurements for the fluorescein-labeled aptamers, the complementary oligonucleotides, and the beacon, were performed using Nanodrop ND3300 fluorospectrometer (Thermo Scientific, Canada). The samples were excited under blue light (470±10 nm), and the emission monitored at 515 nm. All the measurements were recorded in the binding buffer (50mM Tris, 150mM NaCl, 2mM $MgCl_2$ pH, 7.4) at room temperature in triplicate unless otherwise mentioned. The fluorescence spectra presented were the average of three measurements.

2.3 Preparation of Tropomyosin conjugated sepharose beads

Tropomyosin was conjugated with NHS activated sepharose-4B beads according to the protocol as reported previously (H. A. Alhadrami, R. Chinnappan, S. Eissa, A. A. Rahamn, & M. Zourob, 2017). Briefly, sepharose-4B beads were washed with 10-15 volumes of 1mM HCl followed by coupling buffer (50 mM carbonate buffer, pH= 9.2). One mg of TM was added to the slurry of washed sepharose-4B beads and mixed for three hours in 50 mM carbonate buffer (pH= 9.2) at room temperature. The TM conjugated beads were washed with carbonate buffer to remove the unreacted TM. The unreacted active sites in the beads were quenched by treating with 50 mM tris (pH=8) for 1 hour, and then washed with 50 mM Tris, 0.5M NaCl pH=8 and 50 mM sodium acetate, 0.5 M NaCl pH = 4.5 alternatively for 6 times. The washed beads were stored in 10 mM Tris pH = 7.5 and 0.05% sodium azide at 4°C until further use.

2.4 Design of truncated aptamer

As it was assumed the constant primer binding sites of the aptamers are not involved in the formation of aptamer-target complexes, we considered the random sequences of flanked

regions of the aptamers' library for our design. Two different variants of the truncated aptamer have been designed from the secondary structure of the aptamer obtained by m-fold software (at 150 mM NaCl, 2mM $Mgcl_2$ at 25°C). The original parental anti-tropomyosin aptamer sequence was obtained from a previous report (Y. Zhang, Wu, Wei, Zhang, & Mo, 2017). The first aptamer consists of 14 nucleotides (TMT1) and the second variant consists of 26 nucleotides (TMT2) as shown in Fig.1. Both aptamers were labeled with fluorescein at the 5'end and were used for the determination of the dissociation constants and the GO-based biosensors developments.

2.5 Determination of the binding affinity of the truncated aptamer

To find the high affinity truncated aptamer with TM, each aptamer sequence was labeled with fluorescein. The fluorescein-labeled truncated aptamers were heated to 90°C for 5 minutes, 4°C for 10 minutes and room temperature for 10 minutes. Variable concentrations of fluorescein-labeled aptamers (0-250 nM) were incubated with tropomyosin conjugated beads for one hour. The unbound aptamers were removed by washing with binding buffer. The bound DNA was eluted with the elution buffer (7 M urea in 50 mM tris+150 mM +NaCl mM +2 $MgCl_2$). The fluorescence intensities of the eluted DNA from beads were plotted against the concentration of input DNA aptamer. The dissociation constant (K_d) of each aptamers were calculated from the saturation curve by non-linear regression fitting using Prism five software.

2.6 Optimization of Graphene oxide/aptamer ratio and detection

Based on the fluorescence intensity, the concentration of the labeled truncated aptamers has been optimized and used for further experiments. The GO to aptamer ratio were optimized by titrating the 25 nM aptamer with increasing concentration of GO in the rage of 0-50 µg/ml in binding buffer. After the addition of GO to the aptamer solution, the mixture was incubated at room temperature for 30-35 minutes to achieve maximum adsorption of aptamer on the GO

surface. A dynamic range of (0 to 50 µg/ml) of TM in binding buffer was incubated with the mixture of GO-aptamer (15 µg/ml - 25nM) for 30-35 minutes. The fluorescence of each sample was measured with the excitation and emission wavelengths of 470±10 nm and 515 nm respectively. The calibration curve was obtained by plotting the fluorescence intensity changes against the concentration of TM used. The selectivity and cross-reactivity of the biosensor platform were tested with BSA, streptavidin and the analog of recombinant tropomyosin proteins such as prawn (*Penaeus monodon*) recombinant tropomyosin, anisakis (*Anisakis simplex*) recombinant TM and oyster (*Crassostrea gigas*) native TM.

3. Results and Discussion

Aptamers with high affinity towards the target analyte are an essential criterion. Aptamers obtained by conventional SELEX are selected from libraries with 40-60 flanking sequences. Elimination of non-essential nucleotides from unique binding pocket allows for better interaction with target molecules. The constant primer sequences region of the aptamer shows minimal involvement in target binding (Cowperthwaite & Ellington, 2008; Jayasena, 1999). However, the truncation of anti-acetylcholinesterase aptamer showed a significant contribution of the constant primer regions towards binding to the target (Le, Chumphukam, & Cass, 2014). The secondary structure of ssDNA aptamer such as the stem-loop, G-quadruplex, bulges, and pseudoknots in association with the tertiary structures are essential for target binding (Hasegawa, Savory, Abe, & Ikebukuro, 2016; Kaur & Yung, 2012). The recognition of conformational changes in the aptamer upon target binding is critical in biosensor applications. The binding sites of the aptamer may be predicted from the secondary structure followed by the sequence truncation, which could improve the affinity and fabrication of cost-effective biosensor platform (Zhou, Battig, & Wang, 2010).

3.1 Determination of high-affinity aptamer sequence region

Zhang et. al. selected ssDNA aptamer for shrimp TM, by dot-spotting SELEX method, where the dissociation constant of the high-affinity aptamer A5 and A15 are reported as 109 and 127 nM, respectively (Y. Zhang, Wu, Wei, Zhang, & Mo, 2017). Based on the above considerations and m-fold secondary structure prediction, the parental aptamer A15 was truncated into two regions as shown in **Fig. 1.** The secondary structure of anti-TM aptamer consists of 40 nucleotides length excluding the primer binding sites **(Fig.1).** Truncation of the position at site 15 from the 5'end leads to a couple of different short aptamers, where the first one (14 nucleotides) has a stem-loop structure (TMT1) **(Fig.1A).** The second variant (25 nucleotides) has stem-loop and a bulge in the middle of the steam (TMT2) (**Fig.1B)**. In order to compare the reported dissociation constant (K_d) of the parental aptamer, the parent aptamer was fluorescently labeled and incubated with TM conjugated sepharose beads. The K_d was calculated by fitting the saturation curve obtained from the fluorescence intensity against the concentration of the corresponding aptamer **(Fig.2A)**. The obtained K_d value is 115 ± 20 nM which is comparable with the previously reported 124 nM obtained from the enzyme-linked aptamer assay (ELAA) (Y. Zhang, Wu, Wei, Zhang, & Mo, 2017). Similarly, we obtained the plot for the two truncated variants as shown in **Fig.2B**. Saturated fluorescence intensity was not attained as the concentration of TMT1 aptamer increased due to the lack of binding between the TMT1 and TM. Since a very short aptamer (8 nucleotides long) has been reported for a sensitive colorimetric detection approach of tetracycline (Kwon, Raston, & Gu, 2014), we assumed that 14 nucleotides TMT1 aptamer could bind to TM. In other words, this region of the parental aptamer is not essential for the formation of the aptamer-target complex. The dissociation constant, 30 ±15 nM, of TMT2 obtained from the saturation curve reveals that this region of the aptamer is involved in TM binding. Approximately four-fold enhancement in the binding affinity of TMT2 implies that the truncated aptamer may form a unique secondary structure in the presence of $MgCl_2$, and NaCl at the given pH condition,

which ideally allows capturing TM with high affinity. This indicates the major role of TMT2 in the direct binding process and form an aptamer-TM complex. The same aptamer has been used for the development of GO-based fluorescence biosensor for the detection of TM.

3.2 Graphene oxide as a biosensor platform

Graphene oxide (GO) is a two dimensional (2D) aromatic structured material, which consists of multiple layers of graphene sheets. GO is an ideal candidate for the surface adsorption of many biomolecules such as proteins, peptide, DNA, RNA, bacteria and small organic molecules (Huan Zhang, Zhang, Aldalbahi, Zuo, Fan, & Mi, 2017). GO is considered as a much better quencher compared to the usual quencher molecules(Gao, Li, Li, Yan, Zhou, Chen, et al., 2015). GO quenches the fluorescence of the molecules by fluorescence resonance energy transfer (FRET) mechanism. It has been reported that GO interact with ssDNA strongly by non-covalent interactions such as π-π stacking and hydrophobic interactions and quenches the fluorescence of labeled DNA completely (Chinnappan, AlAmer, Eissa, Rahamn, Salah, & Zourob, 2018; Stobiecka, Dworakowska, Jakiela, Lukasiak, Chalupa, & Zembrzycki, 2016). As TMT2 has a higher affinity constant, we used fluorescein labeled truncated aptamer (TMT2) and GO-based fluorescence switching assay for TM detection. This method consists of two steps; GO quenches the fluorescence of the Flu-TMT2 by FRET, in the second step, when the TM was introduced, Flu-TMT2 detach from the GO surface and form Flu-TMT2-TM complex. The amount of TM-aptamer complex suspended in the solution was correlated with the TM concentration through the fluorescence intensity. Therefore, the amount of TM was reflected by the fluorescence intensity as represented in Scheme-1.

3.3 Optimization of GO/Aptamer ratio by fluorescence quenching

The concentration of the Flu-TMT2 aptamer used in this experiment was optimized to be 25 nM, where the fluorescence intensity gave a significant signal to noise ratio (s/n). To get the

minimum GO concentration required for better quenching performance of Flu-TMT2 fluorescence, a fixed amount of aptamer (25 nM) was titrated with a wide range of GO concentration from 0 to 50 µg/ml. In the absence of GO, the Flu-aptamer showed a strong fluorescence intensity as shown in **Fig.3.** Upon addition of increasing concentration of GO, the fluorescence intensity started to decrease and completely disappeared at 35 µg/ml of GO (**Fig 3A, Insert: a - k**). There was no further change in fluorescence intensity with increasing GO concentration which indicates that most of aptamer molecules were stacked on the GO surface and become completely off state. The plot of fluorescence intensity against the concentration of GO shows that 88% quenching at the GO concentration of 15 µg/ml was attained **(Fig.3B).** The ratio of 25 nM of TMT2 for 15 µg/ml of GO has been used as the optimal off state for the rest of the experiments.

3.4 Graphene oxide based aptasensor for Tropomyosin detection

The graphene oxide-based biosensor platform for the detection of TM was established as before (GO/aptamer ratio). As discussed, the full-length aptamer was used for the detection of tropomyosin using GO and DNA intercalating fluorescent dye as a probe. Herein the fluorescent truncated aptamer was used as a recognizing element. As shown in **Fig.4**, the fluorescence of GO-aptamer increases linearly by addition of increasing concentration of TM from 0 to 50 µg/ml. Tropomyosin triggered the fluorescence signal by detaching the aptamer from the GO surface to the solution state. The fluorescence intensity of the released aptamer was plotted against the concentration of TM to generate a calibration curve to determine the detection limit (LOD), and linearity as shown in **Fig.4B**. The LOD was calculated as 3.3 SD/s, where SD is standard deviation of the signal of blank samples and s is the slope of the linear calibration curve. The calculated LOD suggested that the TMT2 aptamer is more sensitive compared to the original aptamer. TMT2 has the LOD of 2.5 nM, which is two-fold higher in sensitivity than the previously reported LOD for the full length aptamer sequence

298 (Y. Zhang, Wu, Wei, Zhang, & Mo, 2017). The enhancement in the LOD is presumably due
299 to the elimination of the non-essential nucleotides from the full-length aptamer which may
300 not be involved in the formation of the binding pocket essential for capturing the target.
301 Moreover, the presence of these non-binding nucleotides enhances the secondary and tertiary
302 structure of the aptamer, which reduces the chance of binding to TM. The LOD of this novel
303 GO-based aptasensor is comparable with the previously reported results generated by
304 expensive and sophisticated instruments. For example, the TM extracted from whole shrimp
305 was estimated by ELISA method with LOD of 1 ppm (1-2 µg/g) (Fuller, Goodwin, & Morris,
306 2006). A sandwich ELISA was reported for the detection of kuruma prawn TM using CE7B2
307 monoclonal antibody with the sensitivity of 90 pg/ml(Hong Zhang, Lu, Ushio, & Shiomi,
308 2014). Werner et al. repotred the detection of TM from various food matrices , had an LOD
309 of 1.6 µg/g which is comparable to our method (Werner, Fæste, & Egaas, 2007). Tabrizi et al
310 developed a photoelectrochemical method for the detection of TM using graphitic carbon
311 nitride and titanium dioxide as photoactive materials with LOD of 0.23 ng/g (Amouzadeh
312 Tabrizi, Shamsipur, Saber, Sarkar, & Ebrahimi, 2017; Werner, Fæste, & Egaas, 2007). This is
313 a relatively more sensitive method compared to our method. However, the fabrication of the
314 photoelectrochemical sensor is a multistep process that involve the utilization of several
315 sophisticated instruments such as SEM, TEM, AFM, X-ray, FTIR etc. Real time PCR (RT-
316 qPCR) based assay was reported for the detection of shrimp TM allergen using shrimp
317 tropomyosin gene. Though the detection limit is 3.2 pg, the method is associated with
318 sophisticated thermos cycler for RT-qPCR amplification(Kim, Kim, Kim, Suh, & Kim,
319 2019). In addition, it is a time-consuming process and requires well trained technicians
320 compared to GO-based fluorometric aptasensor that has been developed in this study.
321

322 **3.5 Selective binding of aptamer**

The binding selectivity between TM and TMT2 was determined by examining the sensor with closely related as well as not related proteins. The cross reactivity of the sensor was evaluated with the allergen TM purified from tiger prawn (*penaeus monodon*), Aniskis (*Aniskis simplex*), and oyster (*Crassostrea gigas*) muscle. In addition, the cross reactivity was examined with other non-related proteins such as streptavidin and BSA as shown in **Fig.5.** TMT2 aptamer cross reacted with recombinant tropomyosin from prawn and Oyster with 80% and 75%, respectively, while 50% cross reactivity was observed with Aniskis recombinant TM. Other proteins such as streptavidin and BSA demonstrated less than 25% cross reactivity with TMT2 aptamer. The amino acid sequences of crustacean TM has a more conservative region in different species, which increases the cross reactivity with the truncated aptamer. In this study, tropomyosin from prawn (crustacean), oyster (mollusc) and anisakis (seafood parasitic nematode) were tested. Oyster and anisakis TMs have 62% and 72% amino acid sequence identity respectively with TM from shrimp. As TMT2 can recognition TM recombinants, it can also be used as a recognising element for detection of TM in various seafood species. Eventually, the GO-TMT2 fluorescence biosensor will be an ideal platform for the detection of recombinant and purified naturally occurring major seafood allergen.

3.6 Validation of GO-aptamer sensor with spiked samples

The developed sensor has been validated with chicken noodle soup obtained from the local market after spiking with shrimp TM. The original soup was diluted 10 times and spiked with TM at a final concentration of 2, 5 and 10 µg/ml. The TM extraction recovery of the spiked samples are summarised in Table-S1 (in the supporting information). The recovered amount of TM that has been measured with the new developed sensor was concordant (close to 100% recovery) with the spiked amount, supporting the reliability of the sensor for detecting TM in food preparations.

4. Conclusions

In summary, a high affinity binding pocket of the aptamer was probed in this study, which can bind to invertebrate TM. The non-essential part of the sequence of the suggested aptamer was truncated for better binding to the target molecule and the affinity increased four-folds after this truncation. The affinity of the fluorescent aptamer was high enough to be used for the development of the GO-based fluorescence assay. The sensitivity of the method increased more than two folds with LOD of 2.5 nM compared to the full-length aptamer sequence. This method was highly selective to shrimp TM. Nevertheless, a significant cross-reactivity was observed to TM of other invertebrate species due to the overall high similarity in their protein amino acid sequences. Finally, the detection and high recovery of TM in chicken soup spiked with TM, support the potential of this sensor to be applied in food analysis. Though, the inhomogeneous consistency of food products is the major challenge for implementation of this method, diluting the sample can eliminate this problem. This method could detect TM of not only shellfish, but can also be used for the detection of different TM allergens from various food sources using specific aptamers. Moreover, the high sensitivity and simplicity will allow multiple target detection using different fluorophore labelled aptamers.

Figure Captions

Scheme.1: Schematic diagram represents fluorescence switching in graphene oxide based fluorescence assay for Tropomyosin

Fig.1. (A) Secondary structure of full-length TM aptamer. (B) Secondary structure 14 nucleotide truncated aptamer (TMT1). (C) Secondary structure of 25 nucleotide truncated aptamer (TMT2).

Fig. 2. Saturation binding affinity curve obtained from the titration of TM with TM-conjugated sepharose beads. (A) Full-length aptamer, (B) Truncated aptamers (TMT1 and TMT2). The error bars represent standard deviation obtained from three different measurements. The fluorescence spectra were recorded by exciting at 470 ±10 nm and the emission intensity 515 nm used for the plots. The error bars represent the standard deviation of three different measurements.

Fig.3. (A) Fluorescence intensity of TMT2 (25nM) with increasing concentration of graphene oxide (GO) a: 0, b: 5, c: 10, d:15, e: 20, f: 25, g: 30, h: 35, I: 40, j: 45, k: 50 µg/ml. (B) Plot GO concentration vs Fluorescence intensity of TMT2 at 515 nm (λ-max). The fluorescence spectra were recorded by exciting at 470 ±10 nm and the emission intensity 515 nm was used for the plots. The error bars represent the standard deviation of three different measurements.

Fig.4. (A) Aptamer release from the GO surface by the addition of (natural tropomyosin from black tiger prawn) TM: Increase in the fluorescence intensity with increase in the concentration of protein : a: 0, b: 0.01, c: 0.1, d: 0.2, e: 0.5, f: 1, g: 2, h: 5, i: 10, j: 20, k: 50 µg/ml. (B) The calibration plot fluorescence intensity against TM concentration (µg/ml) the of TMT2 at 515 nm. The fluorescence spectra were recorded by exciting at 470 ±10 nm and

the emission intensity 515 nm was used for the plots. The error bars represent the standard deviation of three different measurements.

Fig.5. The cross-reactivity response of GO- TMT2 aptamer (15 µg/ml-25 nM) fluorescence assay for different tropomyosin protein samples and irrelevant samples with the concentrations of 5 µg/ml. TM Proteins: Black Tiger Prawn Tropomyosin (BTP TM), Oyster TM, Anisakis TM, Prawn TM and irrelevant protein: BSA and Streptavidin. The fluorescence intensity of each sample obtained by exciting at 470 ±10 nm and the emission intensity 515 nm was used for the plots. The error bars represent the standard deviation of three different measurements.

Scheme 1.

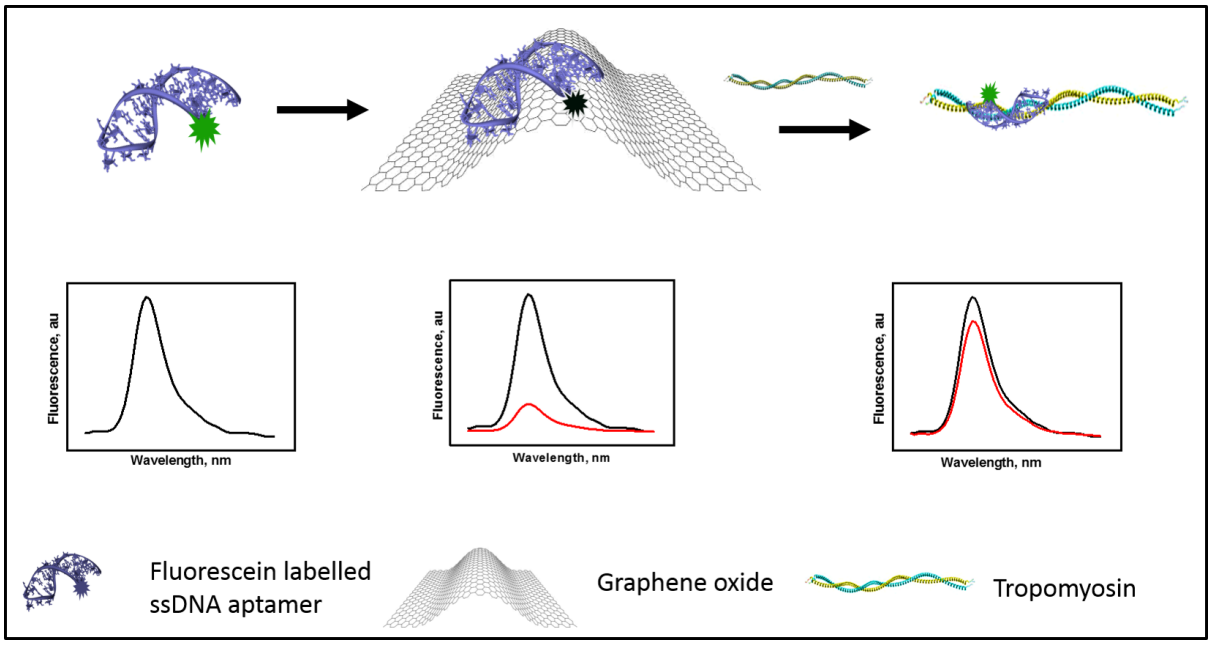
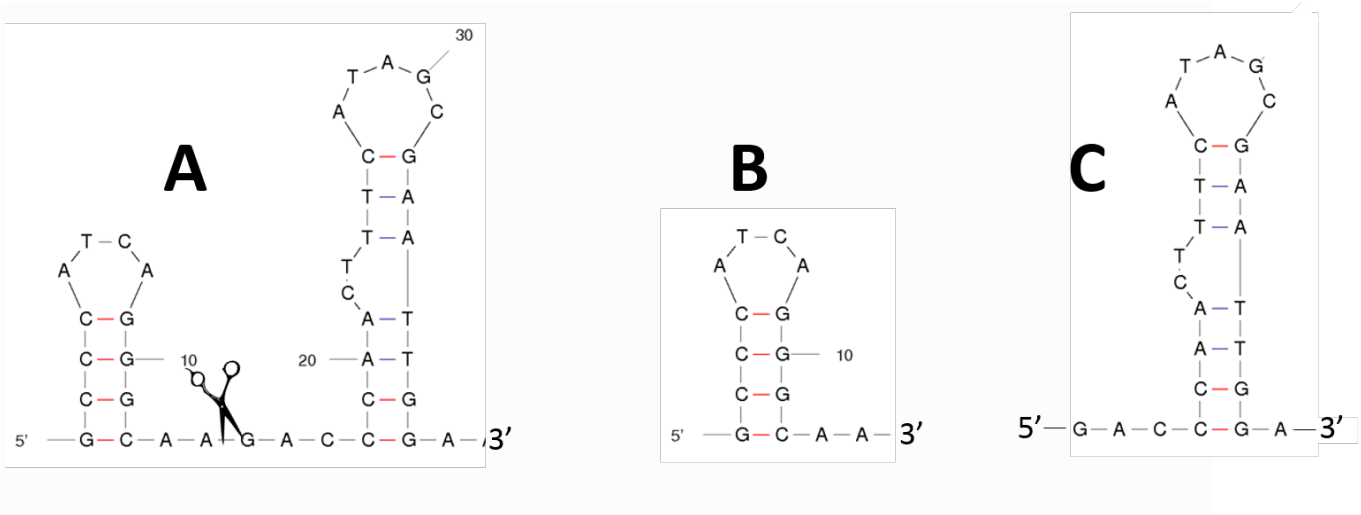

Fluorescence, au
Wavelength, nm
Fluorescence, au
Wavelength, nm
Fluorescence, au
Wavelength, nm
Fluorescein labelled
ssDNA aptamer
Graphene oxide
Tropomyosin

Fig. 1



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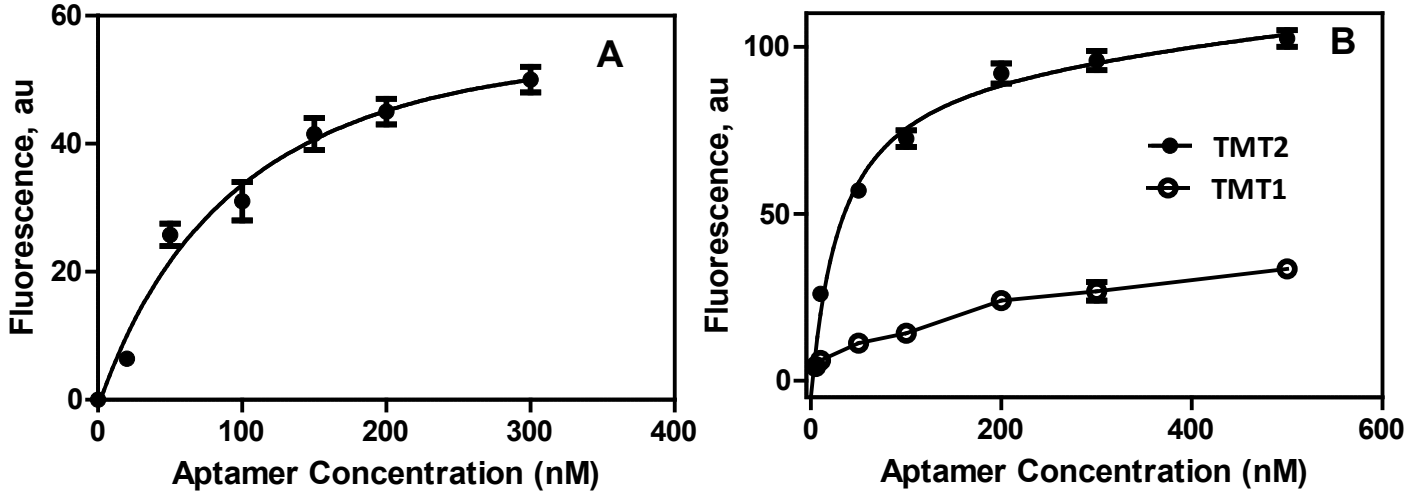
449 **Fig. 2**



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455 **Fig. 3**

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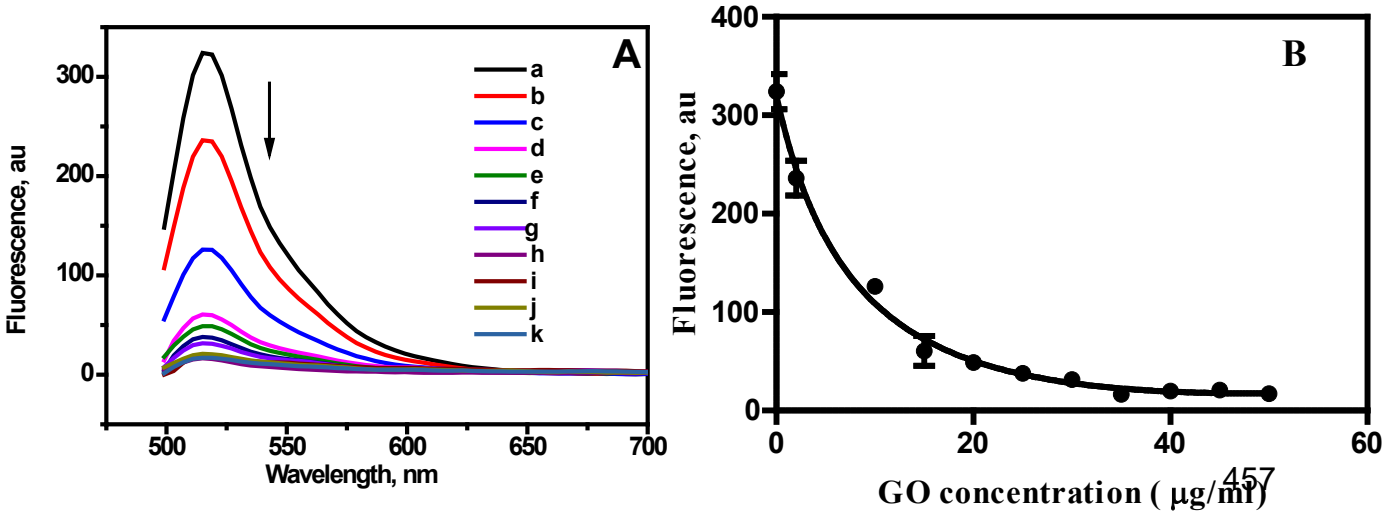

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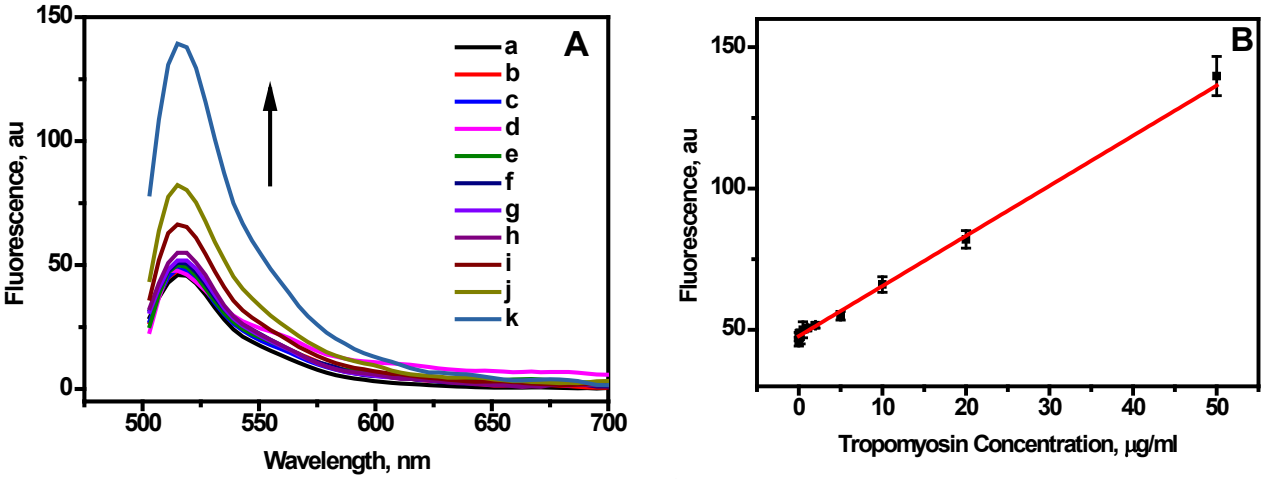
466 **Fig. 4**



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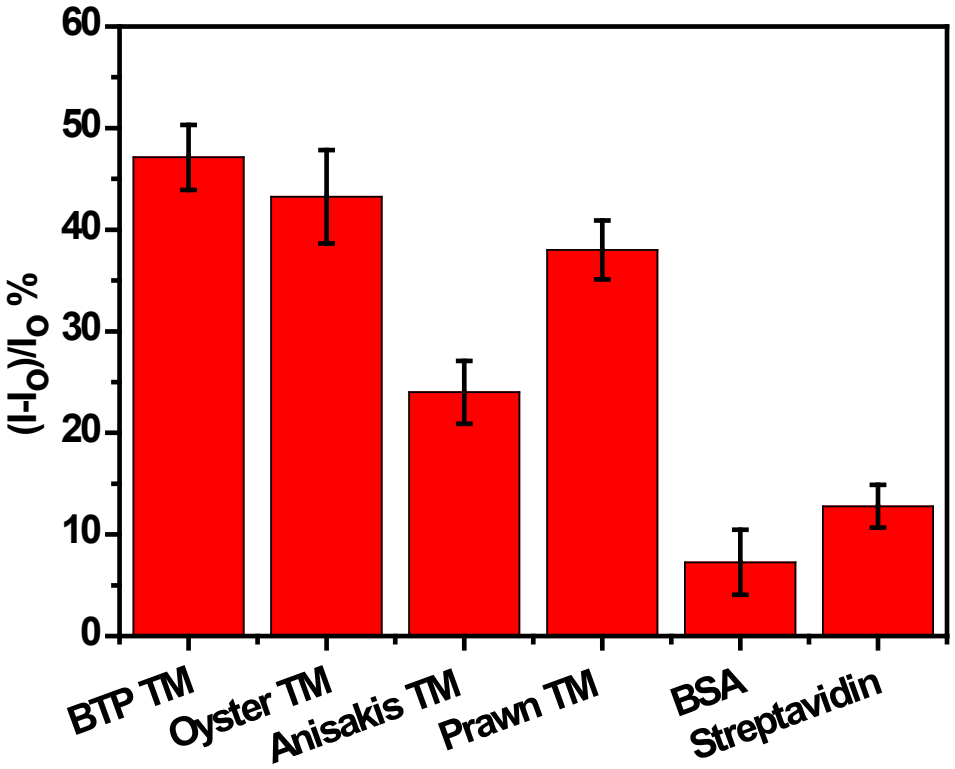
477 **Fig. 5**



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