



Aptamer based fluorometric β -lactoglobulin assay based on the use of magnetic nanoparticles and carbon dots

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Abstract

The authors describe a fluorometric aptamer based assay for detecting β -lactoglobulin by using carbon dots (C-dots) as a signal indicator. The aptamer was immobilized on magnetite (Fe_3O_4) nanoparticles (MNPs), and the C-dots served as a label for the complementary oligonucleotide (cDNA). The assay is based on the hybridization that takes place between aptamer and cDNA. In the presence of β -lactoglobulin (β -LG), the aptamer preferentially binds to β -LG, and this leads to a partial release of the C-dots-cDNA into the solution. After magnetic separation, the supernatant of the solution contains the released C-dots-cDNA which are quantified by fluorometry, best under excitation/emission wavelengths of 354/447 nm. Under the optimal conditions, the fluorescence intensity is proportional to the logarithm of the β -LG concentration in the 0.25 to 50 ng mL⁻¹ range, with a 37 pg mL⁻¹ detection limit. The method was successfully applied to the determination of β -LG in hypoallergenic formulations, and the results demonstrated that this assay is a promising tool in food quality control. Conceivably, it also provides the opportunity for detection of other analytes.

Keywords Fluorescence · Carbon dots · Aptamer · β -LG · Magnetite nanoparticles · Complementary oligonucleotide · DNA hybridization · Magnetic separation · Milk protein allergy · Hypoallergenic formulas

Introduction

Food allergy has become one of the major health concerns nowadays [1]. Among which, cows' milk protein allergy, the most common allergy in early childhood, is estimated to affect 2–3% of infants in their first years [2]. Bovine β -lactoglobulin (β -LG) has been described as the main allergenic protein in cow's milk, which can promote allergic reactions even when present at low concentrations. The symptoms of cows' milk allergy, such as gastrointestinal alterations, atopic dermatitis, urticaria, allergic rhinitis, asthma, angioedema, or chronic cough have been described [3]. In order to diminish the incidence of cows' milk allergy, hypoallergenic formulas (HF) have been commercialised as substitutes for milk. These

formulas are usually prepared from bovine whey proteins and/or caseins that are heat treated and/or hydrolysed with enzyme or acid to eliminate their allergenic character [4]. Unfortunately, in spite of the very low residual amounts of β -LG expected to be present in these HF, allergic reactions have been observed in some instances in infants fed on these formulas. Therefore, it is necessary to establish a method capable to determine the amount of β -LG present in HF to assess their safety. Although several analytical methods have been reported for β -LG detection, including HPLC [5], methods based on the surface plasmon resonance [6, 7], electrochemical immunoassay [8, 9], and fluorescent immunoassay [10]. Among these analytical methods, fluorescent bioassays have been perceived to be one of the most promising analytical tools with wide applicability in various fields because of their fast response, low cost, simple use, as well as high sensitivity.

As a class of novel fluorescent nanomaterials, carbon dots (C-dots) have attracted tremendous attentions owing to their captivating properties such as excellent photostability, low toxicity, favorable biocompatibility and good water solubility [11, 12]. Owing to their outstanding physical and chemical properties, C-dots have been utilized for the construction of novel assays for the detection of protein [13, 14], metal ions

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[15, 16], and other small molecules [17, 18]. However, the development of fluorescence assay is limited for the lack of selectivity despite possessing excellent optical performances. In order to overcome this drawback, aptamers are introduced to the fluorescence assay.

Aptamers, selected from random-sequence nucleic acid libraries by exponential enrichment (SELEX) process [19], are DNA or RNA oligonucleotides, which can specifically bind to target molecules [20]. Aptamers possess great advantages over antibodies, such as low-cost, high stability, easy synthesis and labeling [21]. There are many aptamer based assays developed for the selective sensing of a variety of target molecules [22, 23]. Shima Eissa and co-workers have reported that the 58-mer β -LG aptamer bound to both β -LG A and B protein variants with dissociation constants of 82 and 80 nM, respectively [24]. To the best of our knowledge, an aptamer based fluorometric β -lactoglobulin assay has not yet been reported.

Herein, we established a novel fluorometric assay by using C-dots as a signal indicator and Fe_3O_4 MNPs as a magnetic separator [25] for the detection of β -LG. The aptamer modified Fe_3O_4 MNPs and the complementary oligonucleotide modified C-dots were hybridized to form Fe_3O_4 -aptamer/cDNA-C-dots nanocomposites. In the presence of β -LG, β -LG bound successfully to the aptamer of the nanocomposites with the subsequent release of some C-dots into the solution. The fluorescent signal of the supernatant collected through magnetic separation became stronger with the increase of β -LG concentration. On these grounds, the method built was successfully applied to the detection of trace β -LG in HF.

Materials and methods

Materials and reagents

N-(3-dimethylaminopropyl)-Nethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), Citric acid monohydrate, ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), sodium hydroxide, sodium acetate, ascorbic acid (AA), bovine serum albumin (BSA), glucose (Glu), galactose (Gal), mannose (Man) and fructose (Fru) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China, <http://www.sinoreagent.com/>). Diethylene triamine, trisodium citrate dihydrate, hydrochloric acid and acetone were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China, <http://lingfenghx.cn.makepolo.com/>). Ethylene glycol was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China, <http://www.aladdin-e.com/>). β -LG, casein and γ -globulin were purchased from Shanghai Yuanye Biotechnology Co. Ltd. (Shanghai, China, <http://www.shyuanye.com/>). The purified water used in the study was prepared using Direct-Q water purification system (Millipore, USA, <http://millipore.bioon.com.cn/>). Phosphate buffered saline (PBS) of pH 6.0 and 7.5 contained 10 mM

$\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ and 137 mM NaCl. All reagents were of analytical grade and without any further purification. The DNA oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China, <http://www.sangon.com/>) and their base sequences are given as follows:

β -LG aptamer:

5'-NH₂-(CH₂)₆-CGACGATCGGACCGCAGTAC
CCACCCACCAGCCCCAACATCATGCCCATC
CGTGTGTG-3'

cDNA (complementary to part of the β -LG aptamer):

5'-NH₂-(CH₂)₆-CACACACGGATGGGCATGAT
GTTGGGGCT-3'

Apparatus

FT-IR spectra were recorded on a TENSOR 27 spectrometer (Bruker, Germany, <https://www.bruker.com/>) with a resolution of 2 cm⁻¹ and a spectral range of 4000–400 cm⁻¹. The fluorescence spectra were recorded with an F-4600 fluorescence spectrophotometer (Hitachi, Japan, <http://www.hitachi.com/>). UV–vis absorption spectra were measured with a UV2450 spectrometer (Shimadzu, Japan, <https://www.shimadzu.com.cn/>). The transmission electron microscopic and high-resolution transmission electron microscopic (HRTEM) images were acquired on a JEOL JEM-2100 (HR) transmission electron microscopy (Tokyo, Japan, <https://www.jeol.co.jp/en/products/detail/JEM-2100F.html>). cDNA combined on the surface of C-dots was confirmed by gel electrophoresis (Shanghai Tanon HE-120, <http://www.biotanon.com/>) in 2.0% agarose gel with 1 × TAE buffer (pH 8.0) at 100 V for 60 min.

Synthesis of carboxyl-functionalized Fe_3O_4 MNPs

Fe_3O_4 MNPs were synthesized by a solvothermal method as previously reported with some modification [26]. Typically, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.54 g) and sodium acetate (1.44 g) were dissolved in ethylene glycol (10 mL), respectively. Afterwards, the sodium acetate solution was added dropwise to $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution with stirring. The mixture was stirred vigorously for 30 min and then sealed in a Teflon-lined stainless-steel autoclave (30 mL). The autoclave was heated at 200 °C and maintained for 8 h, and then allowed to cool to room temperature. The products were washed with ethanol and deionized water for several times, and then dried under vacuum at 40 °C for 12 h. In order to modify carboxyl groups on the surface of Fe_3O_4 MNPs, 0.5 g of the Fe_3O_4 MNPs were dispersed in 100 mL of citric acid monohydrate solution (0.105 g mL⁻¹) under ultrasound for 20 min. After that, the mixture was mechanically stirred for 4 h at room temperature. Finally, the

products were washed with deionized water and ethanol for several times, and then dried under vacuum at 40 °C.

Preparation of C-dots

C-dots were synthesized according to a reported method [27]. Briefly, citric acid monohydrate (1.2 g) and diethylene triamine (600 μL) were added into 20 mL of ultrapure water. Then the mixture was transferred to a Teflon-lined autoclave (30 mL) and heated at 200 °C for 4 h. When cooled down to room temperature, the products were concentrated by rotary evaporation, followed by purification with acetone for three times and dried under vacuum overnight at 50 °C.

Preparation of oligonucleotide conjugated nanoparticles

Fe_3O_4 MNPs were first dissolved in PBS (pH 6.0) to obtain Fe_3O_4 MNP solution (0.2 mg mL^{-1}). Then, a mixture of EDC (19 mg) and NHS (22 mg) was added into the solution to activate the carboxyl groups on the surface of Fe_3O_4 MNPs at room temperature under stirring treatment for 30 min, followed by adjusting the pH value to 7.5. The amino-

modified aptamer solution of 100 μM was heated at 95 °C for 4 min, cooled on ice for 4 min and rested at room temperature for a while to facilitate its folding into the minimum energy structure, which is responsible for specific binding. Afterwards, 5 μL of the above pretreated aptamer solution was added into the activated Fe_3O_4 MNP solution to conduct the condensation reaction between the carboxyl and amino groups overnight at room temperature with continuous stirring. Unreacted free aptamer were discarded using magnetic separation. Finally, aptamer-functionalized Fe_3O_4 MNPs were dispersed in PBS (pH 7.5) and stored at 4 °C.

In a similar way, amine-modified cDNA was covalently conjugated onto the carboxyl-functionalized C-dots through EDC/NHS method. The final products C-dots-cDNA were also dispersed in PBS (pH 7.5) and stored at 4 °C.

Procedure for the detection of β -LG

250 μL of Fe_3O_4 -aptamer and 250 μL of C-dots-cDNA were mixed, and then shaken for 2 h at 37 °C with the help of a reciprocating oscillator. After magnetic separation and washing, the Fe_3O_4 -aptamer/cDNA-C-dots bioconjugates were redispersed in 500 μL of PBS (pH 7.5). Afterwards, a

Scheme 1 Schematic illustration of the fabrication of the bioconjugates (a) and working principle for β -LG assay (b)

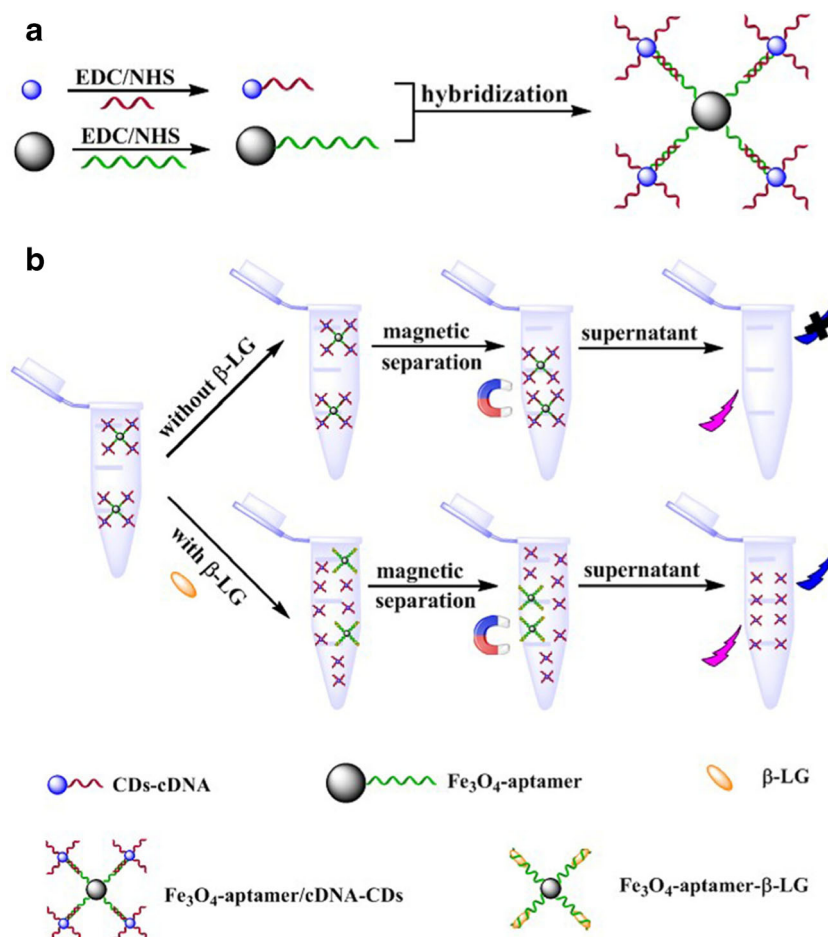
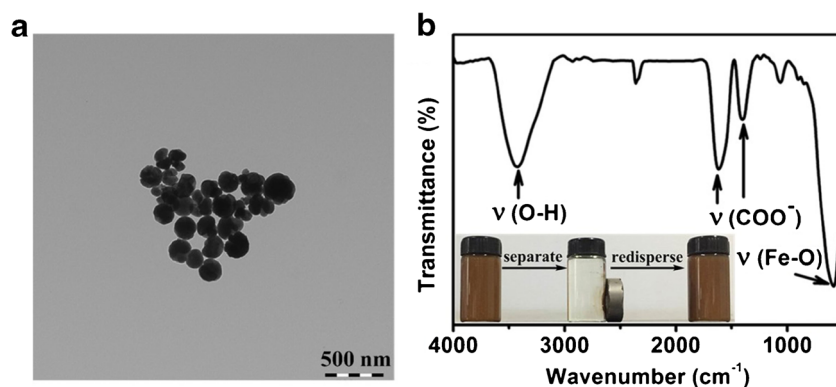


Fig. 1 TEM image (a) and FT-IR spectrum (b) of the carboxyl-functionalized Fe_3O_4 MNPs. Inset: photograph of the Fe_3O_4 MNPs in the absence and presence of an external magnetic field



500 μL of β -LG-containing solution was added to the Fe_3O_4 -aptamer/cDNA-C-dots bioconjugates. After incubation at room temperature for 60 min, the released C-dots were then collected by magnetic separation and their fluorescence intensities were recorded accordingly.

Real sample preparation and measurements

A sample of a commercial HF (Nutrilon pepti®, Nutricia) was prepared as a suspension of 200 mg mL^{-1} in PBS (pH 7.5). The aqueous suspension was manually skimmed after heated at 40 $^{\circ}\text{C}$ for 30 min, centrifugated and cooled for 15–20 min in an ice-bath. The skimmed suspension was subsequently acidified to pH 4.6 with 2 M HCl. After 20 min, the acidified suspension was centrifuged to sediment the caseins. The supernatant was collected and filtered through a 0.2 μm membrane. Finally, 1 M NaOH was employed to neutralise the solution. The determination of β -LG was performed as described in Procedure for the detection of β -LG.

Results and discussion

The principle of β -LG detection

The fabrication of the bioconjugates and detection principle of the assay are shown in Scheme 1. Without β -LG, the resulting Fe_3O_4 -aptamer/cDNA-C-dots bioconjugates can be separated

readily by a magnet. Therefore, the supernatant contains no free C-dots, and thus no fluorescence emission can be detected. When β -LG is present, the incubation of β -LG with Fe_3O_4 -aptamer/cDNA-C-dots would trigger β -LG-aptamer binding, and this causes some C-dots-cDNA dissociating from the surface of Fe_3O_4 -aptamer/cDNA-C-dots. The more β -LG molecules in the detection system, the more Fe_3O_4 -aptamer are combined with the target and thus more C-dots-cDNA release into the solution. Therefore, β -LG concentration can be measured according to the fluorescence intensity of C-dots in the supernatant.

Characterization of carboxyl-functionalized Fe_3O_4 MNPs

Figure 1a shows TEM image of carboxyl-functionalized Fe_3O_4 MNPs. The nanoparticles are approximately spherical, and the average size is about 170 nm. The FT-IR spectrum of carboxyl-functionalized Fe_3O_4 MNPs is shown in Fig. 1b. The obvious peak at 581 cm^{-1} corresponds to the stretching vibration of Fe-O bond. The peak at 3423 cm^{-1} corresponds to the stretching vibration of dissociative hydroxy groups. The peaks at 1617 cm^{-1} and 1400 cm^{-1} are assigned to the asymmetric and the symmetric vibrations of COO^- . This indicates that carboxy groups cover the magnetic particles after solvothermal polymerization. The Fe_3O_4 MNPs can be readily dispersed in buffer and subsequently separated from the

Fig. 2 a TEM image of C-dots, the inset image is high-resolution TEM. (B) UV-vis absorption (solid line), excitation (dot line) and emission (dash line) spectra of C-dots. Inset: photographs of the aqueous dispersed C-dots solution under daylight and 365 nm UV irradiation

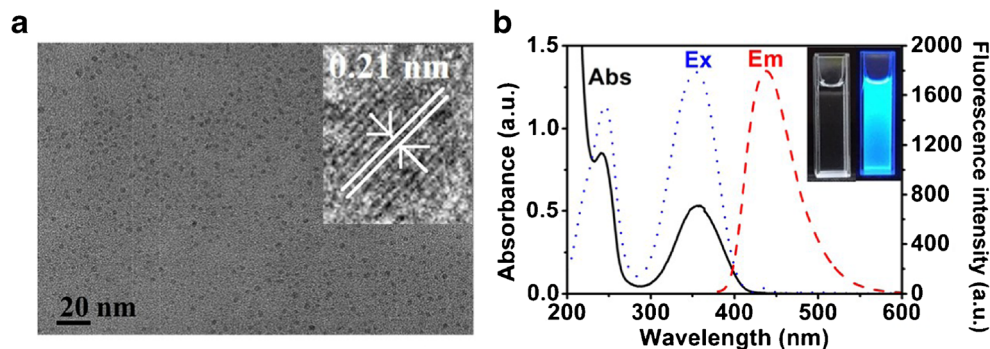
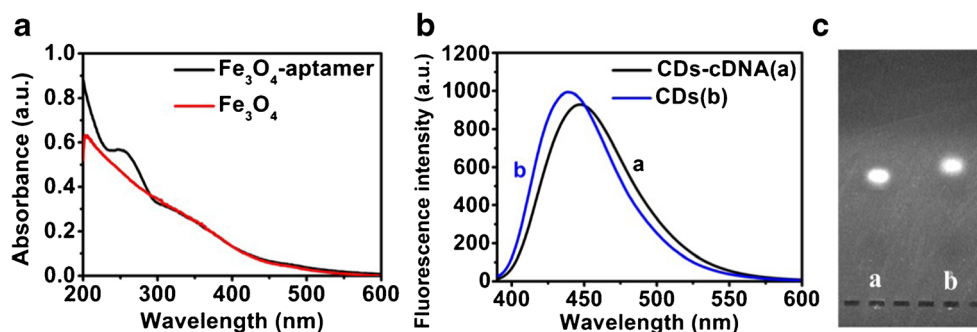


Fig. 3 **a** UV-vis spectra of the plain and functionalized Fe_3O_4 MNPs. **b** Fluorescence spectra of C-dots (b) and cDNA-conjugated C-dots (a). **c** Agarose gel electrophoresis of C-dots-cDNA (a) and cDNA (b)



dispersion within several seconds by an external magnetic field (inset of Fig. 1b).

Characterization of C-dots

As shown in Fig. 2a, the resulting C-dots are relatively uniform in size and exhibit a relatively narrow size distribution between 1.5–3 nm (Fig. S1). The inset in Fig. 2a shows the lattice spacing of 0.21 nm, which corresponds to the [102] facet of graphite [28]. Figure 2b shows the UV-vis absorption spectrum and fluorescence spectrum of the prepared C-dots. It can be seen that the prepared C-dots have absorption peaks at 350 nm and 240 nm, respectively. According to the reference [29], the peak at 240 nm is attributed to π - π^* transition of aromatic C=C bonds, while the absorption at 350 nm is attributed to n - π^* transition of C=O bonds. The FT-IR spectrum of C-dots also proves the existence of carbonyl groups (Fig. S2). The maximum excitation and emission wavelengths of the C-dots are 354 nm and 437 nm, respectively. The aqueous dispersion of C-dots solution exhibits colorless with blue luminescence under 365 nm UV illumination (inset of Fig. 2b).

Characterization of oligonucleotide conjugated nanoparticles

The aptamer and cDNA were covalently attached on the carboxyl terminated Fe_3O_4 MNPs and C-dots, respectively, using

carbodiimide chemistry as described in the experimental section. Plain and functionalized Fe_3O_4 MNPs were characterized using UV-vis spectroscopy as presented in Fig. 3a. The spectrum collected from aptamer functionalized Fe_3O_4 MNPs shows the emergence of characteristic DNA absorption at 260 nm [30], which indicates that aptamer has been conjugated onto the surface of Fe_3O_4 MNPs successfully.

The emission peak of cDNA-conjugated C-dots exhibits a red shift as compared with the fluorescence spectrum of C-dots (Fig. 3b) ($\lambda_{\text{ex}} = 354$ nm). This is mainly due to the covalent binding of cDNA to the C-dots surface and consequent alteration of the C-dots surface charge [31, 32] as well as the size increase of C-dots [33]. For further confirmation, the C-dots-cDNA conjugates were analyzed using agarose gel electrophoresis as presented in Fig. 3c. C-dots-cDNA migrated slower than cDNA due to the increase of mass-to-charge ratio of C-dots-cDNA. This result is corresponding to the previous report [34], proving the successful conjugation of cDNA to C-dots.

Optimization of experimental conditions

The following parameters were optimized: (A) concentration of Fe_3O_4 MNPs; (B) concentration of C-dots; (C) hybridization temperature; (D) hybridization time. Respective experimental data and figures are given in Fig. S3. The following experimental conditions were found to give best results: (A) concentration of Fe_3O_4 MNPs: 0.2 mg mL^{-1} ; (B)

Fig. 4 **a** Fluorescence spectra of the assay ($\lambda_{\text{ex/em}} = 354/447$ nm) in the presence of different concentrations of β -LG (from a to h: 0.25, 0.5, 1, 2.5, 5, 10, 25, 50 ng mL^{-1}). **b** Calibration plot: fluorescence intensity F versus $\log[\beta\text{-LG}]$

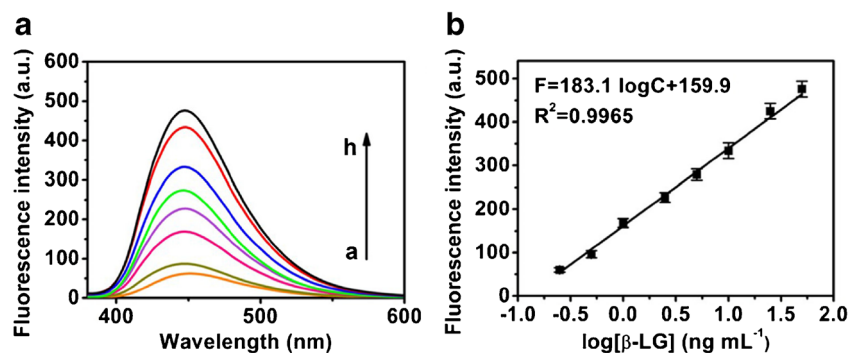
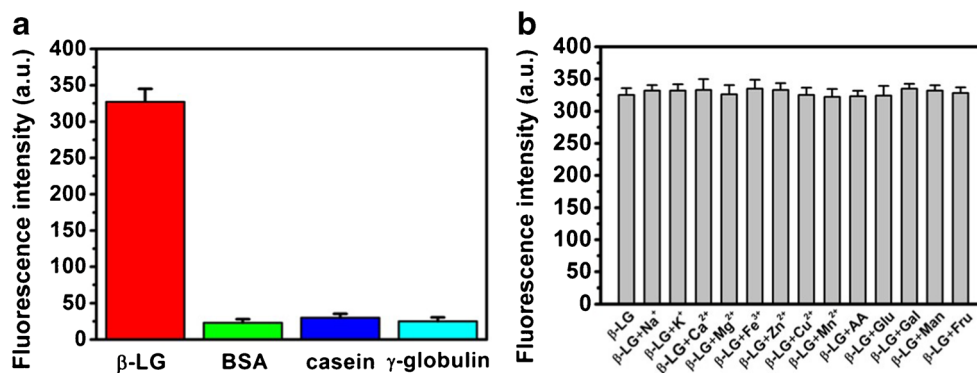


Fig. 5 **a** Responses of the assay to β -LG (10 ng mL^{-1}) and other lactoproteins (100 ng mL^{-1}). **b** Fluorescence intensity of the assay to β -LG (10 ng mL^{-1}) in the absence and presence of other metal ions and interferents: Na^+ , K^+ , Glu, Gal and Man (2 mM), Ca^{2+} (0.2 mM), Mg^{2+} (0.3 mM), Fe^{3+} (0.02 mM), Zn^{2+} (0.01 mM), Cu^{2+} and Mn^{2+} (1 mM), AA (0.1 mM), Fru (0.8 mM)



concentration of C-dots: 0.5 mg mL^{-1} ; (C) hybridization temperature: 37°C ; (D) hybridization time: 2 h.

Analytical performance of the assay

Under the optimized detection conditions, β -LG can be quantitatively detected by observing the fluorescence intensity of the released C-dots in the solution ($\lambda_{\text{ex/em}} = 354/447 \text{ nm}$). The increased fluorescence intensity is directly proportional to the logarithm of β -LG concentration in the range from 0.25 to 50 ng mL^{-1} (Fig. 4a). The calibration curve (Fig. 4b) fits a regression equation of $F = 183.1 \log C + 159.9$ with a correlation coefficient of $R^2 = 0.9965$ (where F is the fluorescence intensity of the released C-dots, C is the concentration of β -LG). The limit of detection of 37 pg mL^{-1} is obtained based on $3\sigma/k$ (where, σ is the standard deviation of blank measurements ($n = 10$), and k is the slope of calibration curve). The analytical performances of the present assay for β -LG detection along with the others reported in literatures are all summarized in Table S1. As can be seen, the assay exhibits a broad linear range and its sensitivity is comparable to the corresponding values observed in most of the works.

Table 1 The intra-day and inter-day precisions of the β -LG determination ($n = 3$)

Concentration of β -LG (ng mL^{-1})	intra-day (ng mL^{-1})	RSD (%)	inter-day (ng mL^{-1})	RSD (%)
0.50	0.47	4.5	0.43	6.6
	0.44		0.49	
	0.48		0.47	
5.00	4.70	3.8	5.20	5.8
	5.07		4.82	
	4.88		5.40	
40.00	37.04	3.2	44.63	5.7
	38.87		41.91	
	39.36		39.87	

Specificity and reproducibility

To evaluate the specificity of the fabricated assay for β -LG, three lactoproteins including BSA, casein and γ -globulin were all investigated. As shown in Fig. 5a, it is obvious that the fluorescence signals to BSA, casein and γ -globulin are neglectable with the concentration of 100 ng mL^{-1} , while an obvious increase in fluorescence signal is observed for β -LG with a concentration of 10 ng mL^{-1} . This result suggests that the assay possesses high selectivity. No obvious interfering effects are observed in the presence of other metal ions and interferents. As shown in Fig. 5b, the fluorescence intensity keeps almost no changed when β -LG is mixed with other metal ions and interferents, compared with that of β -LG alone. The intra-day and inter-day relative standard deviations (RSDs) of this method are all less than 10% (Table 1). These results suggest that the method exhibited has good reproducibility.

Analytical application in real sample

To further investigate the potential practical application of the method, this assay was applied to the determination of β -LG in a HF sample subjected to a simple pre-treatment process. The results are shown in Table 2. According to the reference reported [35], the amount of β -LG in HF is below 0.5 ppm . The result of our detection is coincided with the reference. As can be seen, the average recoveries of the spiked β -LG range from 88.4% to 93.8% with a satisfying analytical precision ($\text{RSD} \leq 6.4\%$). All of the findings indicate the acceptable accuracy

Table 2 Determination of β -LG in hypoallergenic formulas by the fluorescent assay built ($n = 3$)

Sample	Spiked (ng g^{-1})	Found (ng g^{-1})	Recovery (%)	RSD (%)
1	0.00	43.05	—	4.7
2	40.00	78.41	88.4	6.4
3	80.00	115.77	90.9	4.2
4	120.00	155.61	93.8	3.5

and validity of the assay as applied to β -LG detection in real samples.

Conclusion

In this work, a novel aptamer based fluorescent assay for quantification of β -LG in hypoallergenic formulas is reported. To the best of our knowledge, there has never been a report on the application of aptamer based fluorometric β -LG assay until now. The platform exhibits several advantages compared to previous β -LG assays: (1) The aptamer exploited in our approach is easily available and more stable than commonly used antibodies; (2) the C-dots used in this method make our approach more promising for β -LG detection than other methods based on dye-doped fluorescent nanoparticles or quantum dots, owing to the excellent photostability, and the autofluorescence-background-free feature of C-dots; and (3) the introduction of magnetic separation has been found to be effective for β -LG detection. As a result, highly sensitive detection of as low as 37 pg mL^{-1} β -LG as well as satisfactory recoveries ranging from 88.4% to 93.8% in practical samples have been realized, indicating its promising future in dairy products quality control. We believe that this novel strategy provides not only a promising method for β -LG detection but also a universal model for detecting diverse targets in various fields by changing the corresponding aptamer.

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Compliance with ethical standards The author(s) declare that they have no competing interests.

References

- Eissa S, L'Hocine L, Sij M, Zourob M (2013) A graphene-based label-free voltammetric immunoassay for sensitive detection of the egg allergen ovalbumin. *Analyst* 138:4378–4384. <https://doi.org/10.1039/c3an36883a>
- Lifschitz C, Szajewska H (2015) Cow's milk allergy: evidence-based diagnosis and management for the practitioner. *Eur J Pediatr* 174:141–150. <https://doi.org/10.1007/s00431-014-2422-3>
- Exl BM (2001) A review of recent developments in the use of moderately hydrolyzed whey formulae in infant nutrition. *Nutr Res* 21:355–379. [https://doi.org/10.1016/S0271-5317\(00\)00259-1](https://doi.org/10.1016/S0271-5317(00)00259-1)
- Besler M, Steinhart H, Paschke A (2001) Stability of food allergens and allergenicity of processed foods. *J Chromatogr B* 756(2001): 207–228. [https://doi.org/10.1016/S0378-4347\(01\)00110-4](https://doi.org/10.1016/S0378-4347(01)00110-4)
- Ren Y, Han Z, Chu X, Zhang J, Cai Z, Wu Y (2010) Simultaneous determination of bovine α -lactalbumin and β -lactoglobulin in infant formulae by ultra-high-performance liquid chromatography-mass spectrometry. *Anal Chim Acta* 667:96–102. <https://doi.org/10.1016/j.aca.2010.04.015>
- Billakanti JM, Fee CJ, Lane FR, Kash AS, Fredericks R (2010) Simultaneous, quantitative detection of five whey proteins in multiple samples by surface plasmon resonance. *Int Dairy J* 20:96–105. <https://doi.org/10.1016/j.idairyj.2009.08.008>
- Wu X, Li Y, Liu B, Feng Y, He W, Liu Z, Liu L, Wang Z, Huang H (2015) Two-site antibody Immunoanalytical detection of food allergens by surface Plasmon resonance. *Food Anal Methods* 9:582–588. <https://doi.org/10.1007/s12161-015-0232-5>
- Eissa S, Tili C, L'Hocine L, Zourob M (2012) Electrochemical immunoassay for the milk allergen β -lactoglobulin based on electrografting of organic film on graphene modified screen-printed carbon electrodes. *Biosens Bioelectron* 38:308–313. <https://doi.org/10.1016/j.bios.2012.06.008>
- Ruiz-Valdepeñas Montiel V, Campuzano S, Conzuelo F, Torrente-Rodríguez RM, Gamella M, Reviejo AJ, Pingarrón JM (2015) Electrochemical magnetoimmunosensing platform for determination of the milk allergen β -lactoglobulin. *Talanta* 131:156–162. <https://doi.org/10.1016/j.talanta.2014.07.076>
- He S, Li X, Gao J, Tong P, Chen H (2017) Development of a H_2O_2 -sensitive quantum dots-based fluorescent sandwich ELISA for sensitive detection of bovine β -lactoglobulin by monoclonal antibody. *J Sci Food Agric* doi. <https://doi.org/10.1002/jsfa.8489>
- Baker SN, Baker GA (2010) Luminescent carbon nanodots: emergent nanolights. *Angew Chem Int Ed* 49:6726–6744. <https://doi.org/10.1002/anie.200906623>
- Lim SY, Shen W, Gao Z (2015) Carbon quantum dots and their applications. *Chem Soc Rev* 44:362–381. <https://doi.org/10.1039/c4cs00269e>
- Miao H, Wang L, Zhuo Y, Zhou Z, Yang X (2016) Label-free fluorimetric detection of CEA using carbon dots derived from tomato juice. *Biosens Bioelectron* 86:83–89. <https://doi.org/10.1016/j.bios.2016.06.043>
- Wu Y, Wei P, Pengpumiak S, Schumacher EA, Remcho VT (2015) Development of a carbon dot (C-dot)-linked immunosorbent assay for the detection of human α -fetoprotein. *Anal Chem* 87:8510–8516. <https://doi.org/10.1021/acs.analchem.5b02019>
- Zou C, Foda MF, Tan X, Shao K, Wu L, Lu Z, Bahloul HS, Han H (2016) Carbon-dot and quantum-dot-coated dual-emission Core-satellite silica nanoparticles for Ratiometric intracellular Cu^{2+} imaging. *Anal Chem* 88:7395–7403. <https://doi.org/10.1021/acs.analchem.6b01941>
- Zhou Y, Huang X, Liu C, Zhang R, Gu X, Guan G, Jiang C, Zhang L, Du S, Liu B, Han MY, Zhang Z (2016) Color-multiplexing-based fluorescent test paper: dosage-sensitive visualization of arsenic(III) with discernable scale as low as 5 ppb. *Anal Chem* 88:6105–6109. <https://doi.org/10.1021/acs.analchem.6b01248>
- Wang H, Lu Q, Hou Y, Liu Y, Zhang Y (2016) High fluorescence S, N co-doped carbon dots as an ultra-sensitive fluorescent probe for the determination of uric acid. *Talanta* 155:62–69. <https://doi.org/10.1016/j.talanta.2016.04.020>
- Huang Y, Zhou J, Feng H, Zheng J, Ma HM, Liu W, Tang C, Ao H, Zhao M, Qian Z (2016) A dual-channel fluorescent chemoassay for discriminative detection of glutathione based on functionalized carbon quantum dots. *Biosens Bioelectron* 86:748–755. <https://doi.org/10.1016/j.bios.2016.07.081>
- Osborne SE, Ellington AD (1997) Nucleic acid selection and the challenge of combinatorial chemistry. *Chem Rev* 97:349–370. <https://doi.org/10.1021/cr960009c>
- Crivianu-Gaita V, Thompson M (2016) Aptamers, antibody scFv, and antibody Fab' fragments: an overview and comparison of three of the most versatile bioassay biorecognition elements. *Biosens Bioelectron* 85:32–45. <https://doi.org/10.1016/j.bios.2016.04.091>
- Famulok M, Mayer G (2011) Aptamer modules as assays and detectors. *Acc Chem Res* 44:1349–1358. <https://doi.org/10.1021/ar2000293>

22. Shahdost-Fard F, Roushani M (2016) Conformation switching of an aptamer based on cocaine enhancement on a surface of modified GCE. *Talanta* 154:7–14. <https://doi.org/10.1016/j.talanta.2016.03.055>
23. Huang H, Shi S, Gao X, Gao R, Zhu Y, Wu X, Zang R, Yao T (2016) A universal label-free fluorescent aptaassay based on Ru complex and quantum dots for adenosine, dopamine and 17 β -estradiol detection. *Biosens Bioelectron* 79:198–204. <https://doi.org/10.1016/j.bios.2015.12.024>
24. Eissa S, Zourob M (2017) In vitro selection of DNA aptamers targeting β -lactoglobulin and their integration in graphene-based bioassay for the detection of milk allergen. *Biosens Bioelectron* 91:169–174. <https://doi.org/10.1016/j.bios.2016.12.020>
25. Xiao D, Lu T, Zeng R, Bi Y (2016) Preparation and highlighted applications of magnetic microparticles and nanoparticles: a review on recent advances. *Microchim Acta* 183:2655–2675. <https://doi.org/10.1007/s00604-016-1928-y>
26. Deng Y, Qi D, Deng C, Zhang X, Zhao D (2008) Superparamagnetic high-magnetization microspheres with an Fe₃O₄@SiO₂ Core and perpendicularly aligned Mesoporous SiO₂ Shell for removal of microcystins. *J Am Chem Soc* 130:28–29. <https://doi.org/10.1021/ja0777584>
27. Zhu S, Meng Q, Wang L, Zhang J, Song Y, Jin H, Zhang K, Sun H, Wang H, Yang B (2013) Highly photoluminescent carbon dots for multicolor patterning, assays, and bioimaging. *Angew Chem Int Ed* 52:3953–3957. <https://doi.org/10.1002/anie.201300519>
28. Zhang H, Chen Y, Liang M, Xu L, Qi S, Chen H, Chen X (2014) Solid-phase synthesis of highly fluorescent nitrogen-doped carbon dots for sensitive and selective probing ferric ions in living cells. *Anal Chem* 86:9846–9852. <https://doi.org/10.1021/ac502446m>
29. Dong Y, Wang R, Li G, Chen C, Chi Y, Chen G (2012) Polyamine-functionalized carbon quantum dots for chemical sensing. *Carbon* 50:2810–2815. <https://doi.org/10.1021/ac3012126>
30. Jiang Q, Zhang D, Cao Y, Gan N (2017) An antibody-free and signal-on type electrochemiluminescence assay for diethylstilbestrol detection based on magnetic molecularly imprinted polymers-quantum dots labeled aptamer conjugated probes. *J Electroanal Chem* 789:1–8. <https://doi.org/10.1016/j.jelechem.2017.02.020>
31. Alibolandi M, Hadizadeh F, Vajhedin F, Abnous K (2015) Ramezani M design and fabrication of an aptaassay for chloramphenicol based on energy transfer of CdTe quantum dots to graphene oxide sheet. *Mater Sci Eng C* 48:611–619. <https://doi.org/10.1016/j.msec.2014.12.052>
32. Dong H, Gao W, Yan F, Ji H, Ju H (2010) Fluorescence resonance energy transfer between quantum dots and Graphene oxide for sensing biomolecules. *Anal Chem* 82:5511–5517. <https://doi.org/10.1021/ac100852z>
33. Liu H, Xu S, He Z, Deng A, Zhu JJ (2013) Supersandwich Cytoassay for selective and ultrasensitive detection of cancer cells using Aptamer-DNA Concatamer-quantum dots probes. *Anal Chem* 85:3385–3392. <https://doi.org/10.1021/ac303789x>
34. He S, Huang BH, Tan J, Luo QY, Lin Y, Li J, Hu Y, Zhang L, Yan S, Zhang Q, Pang DW, Li L (2011) One-to-one quantum dot-labeled single long DNA probes. *Biomaterials* 32:5471–5477. <https://doi.org/10.1016/j.biomaterials.2011.04.013>
35. Knipping K, van Roest M, Kruijssen L, Smits M, Teunis M, Cox L, de Jong N, Simons PJ, Boon L, Teshima R, Gros M, Kegler D, Garssen J, Knippels LM, Pieters R (2016) Intra- and inter-laboratory validation of an innovative huFc ϵ RI α -RBL-2H3 degranulation assay for in vitro allergenicity assessment of whey hydrolysates. *Toxicol in Vitro* 33:29–34. <https://doi.org/10.1016/j.tiv.2016.02.018>

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Aptamer based fluorometric β -lactoglobulin assay based on
the use of magnetic nanoparticles and carbon dots

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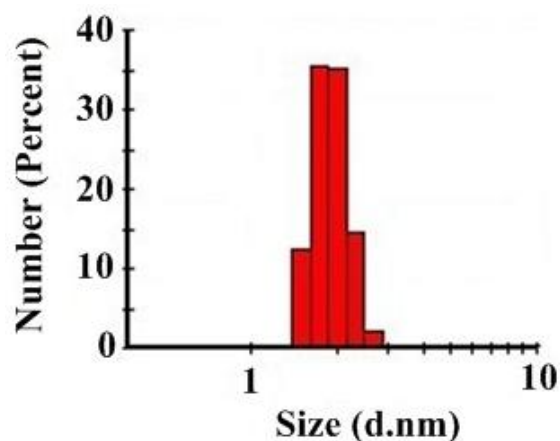


Fig. S1 Size distribution of C-dots

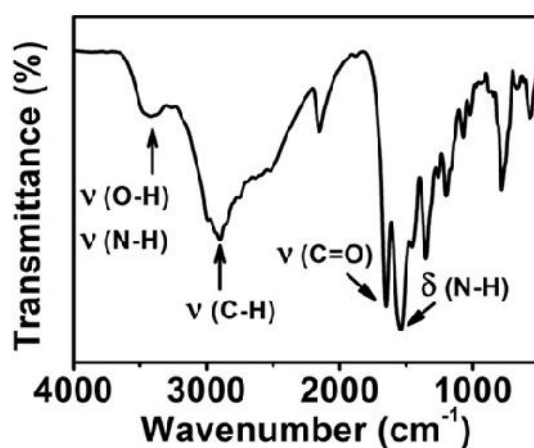


Fig. S2 FT-IR spectrum of C-dots

The absorption peak at 1555 cm^{-1} is attributed to the in-plane bending vibration of -NH. The characteristic peak around 1657 cm^{-1} is attributed to the stretching vibration of C=O. The existence of strong absorption peak of -OH and -NH around 3423 cm^{-1} indicates that there are plenty of carboxyl and amino groups on the surface of C-dots.

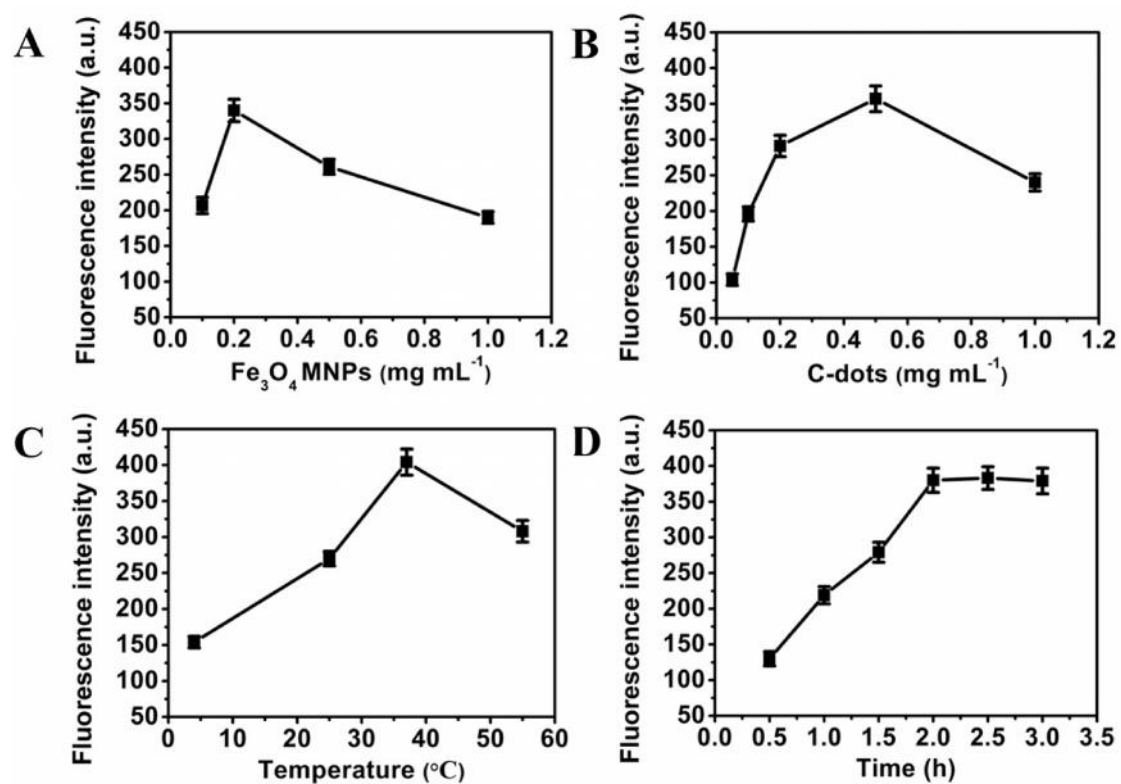


Fig. S3 The effects of different concentrations of Fe_3O_4 MNPs (A) and C-dots (B), hybridization temperature (C) and hybridization time (D) on the fluorescence responses of the aptasensor upon the exposure to 10 ng mL^{-1} of $\beta\text{-LG}$

Table S1 The analytical performances comparison between the proposed method and the other techniques

Methods	Linear range	LOD	Reference
Capillary electrophoresis	5×10^{-10} - 10^{-7} M	5×10^{-10} M	[1]
HPLC	0.4-60 $\mu\text{g mL}^{-1}$	0.06 $\mu\text{g mL}^{-1}$	[2]
Electrochemical magnetoimmunosensing	2.8-100 ng mL^{-1}	0.8 ng mL^{-1}	[3]
Voltammetric immunosensor	0.001-100 ng mL^{-1}	0.85 pg mL^{-1}	[4]
Optical near field biosensor	0.1-100 $\mu\text{g mL}^{-1}$	N/A	[5]
Surface plasmon resonance	5.0-4000 ng mL^{-1}	5.54 ng mL^{-1}	[6]
Colourimetric immunosensor	10- 10^6 ng mL^{-1}	N/A	[7]
Voltametric aptasensor	0.1-100 ng mL^{-1}	20 pg mL^{-1}	[8]
Fluorescent immunoassay	0.48-62.5 ng mL^{-1} and 125-4000 ng mL^{-1}	0.49 ng mL^{-1}	[9]
Fluorescent aptasensor	0.25-50 ng mL^{-1}	37 pg mL^{-1}	This work

References:

1. Pelaez-Lorenzo C, Diez-Masa JC, Vasallo I, de Frutos M (2009) A new sample preparation method compatible with capillary electrophoresis and laser-induced fluorescence for improving detection of low levels of β -lactoglobulin in infant foods. *Anal Chim Acta* 649:202-210. doi: 10.1016/j.aca.2009.07.033
2. Ren Y, Han Z, Chu X, Zhang J, Cai Z, Wu Y (2010) Simultaneous determination of bovine α -lactalbumin and β -lactoglobulin in infant formulae by ultra-high-performance liquid chromatography-mass spectrometry. *Anal Chim Acta* 667:96-102. doi: 10.1016/j.aca.2010.04.015
3. Ruiz-Valdepeñas Montiel V, Campuzano S, Conzuelo F, Torrente-Rodríguez RM, Gamella M, Reviejo AJ, Pingarrón JM (2015) Electrochemical magnetoimmunosensing platform for determination of the milk allergen β -lactoglobulin. *Talanta* 131:156-162. doi: 10.1016/j.talanta.2014.07.076
4. Eissa S, Tlili C, L'Hocine L, Zourob M (2012) Electrochemical immunosensor for the milk allergen β -lactoglobulin based on electrografting of organic film on graphene modified screen-printed carbon electrodes. *Biosens Bioelectron* 38:308-313. doi: 10.1016/j.bios.2012.06.008
5. Hohensinner V, Maier I, Pittner F (2007) A 'gold cluster-linked immunosorbent assay': Optical near-field biosensor chip for the detection of allergenic β -lactoglobulin in processed milk matrices. *J Biotechnol* 130:385-388. doi:10.1016/j.jbiotec.2007.05.020
6. Wu X, Li Y, Liu B, Feng Y, He W, Liu Z, Liu L, Wang Z, Huang H (2015)

Two-Site Antibody Immunoanalytical Detection of Food Allergens by Surface Plasmon Resonance. Food Anal Method 9:582-588. doi:10.1007/s12161-015-0232-5

7. Maier I, Lindner W, Pittner F (2009) Antigenicity of heat-treated and trypsin-digested milk samples studied by an optical immunochip biosensor. Monatsh Chem 140:921-929. doi:10.1007/s00706-009-0148-0
8. Eissa S, Zourob M (2017) In vitro selection of DNA aptamers targeting β -lactoglobulin and their integration in graphene-based biosensor for the detection of milk allergen. Biosens Bioelectron 91:169-174. doi: 10.1016/j.bios.2016.12.020
9. He S, Li X, Gao J, Tong P, Chen H (2017) Development of a H_2O_2 -sensitive quantum dots-based fluorescent sandwich ELISA for sensitive detection of bovine β -lactoglobulin by monoclonal antibody. J Sci Food Agric doi: 10.1002/jsfa.8489.