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Article

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Mast cell-based fluorescence biosensor for rapid detection of major fish allergen parvalbumin

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ABSTRACT

In this study, we developed a rat basophilic leukemia cell (RBL-2H3) fluorescence sensor to detect and identify the major fish allergen parvalbumin (PV). We constructed and transfected a CD63-enhanced green fluorescent protein (EGFP) plasmid into RBL cells through a highly efficient, lipid-mediated, DNA-transfection procedure. Stable transfectant RBL cells were then obtained for a cell fluorescence assay with confocal laser scanning microscopy. Results show the cell surface expression of CD63 reflects degranulation, indicating that a fluorescence assay with these cells could efficiently measure the activation of antigen-stimulated transfectant cells and detect antigens with a nanogram level. Therefore, this cell-based fluorescence biosensor technique for detecting fish PV exhibits promise for quantifying fish PV after anti-PV immunoglobulin E (*Ig*E)-stimulation. Results show that fluorescence intensities increased with purified PV concentrations from 1 to 100 ng/mL, with a detection limit of 0.35 ng/mL (RSD 4.5%), confirmed by *β*-hexosaminidase assays. These RBL mast cells transfected with the CD63-EGFP gene, and responded to PV only when they were sensitized with the specific *Ig*E antibody. This demonstrates the utility of this highly sensitive biosensor for food allergen detection and prediction.

Key words: Fluorescence sensor, RBL-2H3 mast cells, fish parvalbumin, CD63, green fluorescent protein, confocal laser scanning microscopy

■ INTRODUCTION

Fish represents an important constituent of the human diet. However, some species may cause severe allergic reactions even in trace quantities that make fish allergies among the most common food allergies. [1] Nowadays with the mass consumption of fish in all parts of the world, the growth of population infected with fish hypersensitivity has accelerated dramatically[2] A recent U.S. survey reported that ~0.4% of the general population have fish allergies, in which nearly 40% of children are sensitized to fish. [3]

Stimulation with fish allergens can trigger severe Immunoglobulin E (IgE)-mediated allergic diseases, such as urticaria, diarrhea, and dyspnea. [4] Fish allergenicity is mainly related to the expression concentration of parvalbumin (PV), the major allergen in fish. [5]. This ~12 kDa protein has been commonly found in all fish sarcoplasmic proteins with characteristics of high water solubility, well stability and an isoelectric point of 4. Moreover, PVs are believed to be the only major allergens for 95% of the patients affected with IgE-mediated fish hypersensitivity. [6]

Currently, a number of technical approaches have been developed to identify fish PV in food, including chromatography, [7] mass spectrometry, [8] real-time PCR, [9] and Protein Chip,[10] and ELISA, [11,12,1] However, none of these detection methods is perfect, each method has its own drawbacks such as long detection time, complex sample pretreatment and complicated interpretation of results. Hence, there is a need for a rapid and visible tool to accurately quantify fish PVs as a new attempt and supplement.

Recently, living cell-based sensors have provided a new strategy for in vitro tests. Sensors for detecting target antigens are developed by a specific biological recognition element and the identification process can be converted into a recordable and quantitative signal.[13] Therefore, cell-based biosensors have been widely employed in many fields, including drug screening, environmental test, medical diagnosis and national security.[14-17] Utilizing the natural ability of mast cells to identify antigens is a stable and accurate strategy that mimics physiological conditions.[18,19] Because, there are abundant high-affinity receptors (FcεRI) on the surface of Rat basophilic leukemia (RBL) cells, to which IgE antibodies can tightly bind. Subsequent crosslinking of FcεRI/IgE complexes causes cell degranulation and release of intracellular inflammatory mediators that initiates the antigens-recognition ability of these pre-sensitized cells. Hence, such a cell sensor can respond to very low antigen concentrations with a rapid response. [20]

However, traditional methods for measuring the release of cell inflammatory mediators are time-consuming, require complex sample pretreatment procedures, and the entire reaction process of cell sensitization cannot be directly observed. [21] To address these issues, this study constructed and transfected a plasmid for the expression of CD63-enhanced green fluorescent protein (EGFP) fusion protein into RBL mast cell nuclei. This gave these cells the ability to express fluorescence spontaneously when exposed to an allergen antigen. CD63 glycoprotein can be easily found on basophilic granular membranes in many cells such as basophils, mast cells, and platelets. [22, 23] The integration of cytoplasmic granules with the plasma membrane

occurs when the of basophils or mast cells are activated along with the successive release of inflammatory mediators, such as histamine.[24, 25] Although, CD63 antigen expression on mast cell surfaces has been always detected by flow cytometric analysis.[26] However, the real-time expression of CD63 in individual cells cannot be observed.[27] Therefore, this study employed confocal fluorescence microscopy to examine CD63 surface expression on RBL mast cells stimulated with antigen, resulting in a rapid quantification method for fish PVs.

This study developed a novel, mast cell-based, fluorescent sensor to quantify fish PV and evaluate IgE-mediated hypersensitivity. We contructed and transfected CD63-EGFP plasmid into RBL cells, to obtain stable genetically fluorescent cell lines. We also explored the use of the real-time surface expression of CD63-EGFP in anti-DNP IgE pre-sensitized RBL-2H3 cells exposed to DNP-BSA as a model for fluorescence detection by confocal laser scanning microscopy (CLSM). We measured changes in fluorescence intensity induced by the fish PV on mast cells and analyzed the relationship between different allergen concentrations and impedance signals. These RBL cell sensors successfully detected and identified target allergens within minutes, and the allergic reaction process was monitored in real-time by CLSM.

■EXPERIMENTAL PROCEDURES

Reagents and chemicals. Mouse monoclonal anti-dinitrophenyl IgE antibody (DNP-IgE), dinitrophenyl-BSA (DNP-BSA), sulforhodamine B (SRB), bromochloroindolyl phosphate, nitro blue tetrazolium, and trichloroacetic acid were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Purified fish PV and mouse monoclonal anti-fish PV IgE antibody was purchased from Novus Biologicals

(Littleton, CO, USA). Rat basophilic leukemia (RBL) cells were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). RPMI 1640 medium and fetal bovine serum (FBS) was obtained from Gibco Laboratories (Gaithersburg, MD, USA). Thirty-five-mm glass-bottomed dishes were purchased from Shengyou Biotechnology Co., Inc. (Hangzhou, China). Other conventional reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All solutions were prepared with deionized water and all reagents analytical grade.

Instrumentation Fluorescence signals were recorded by CLSM (LSM 710, Carl Zeiss Microscopy GmbH, Göttingen, Germany). A FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA) was used to evaluate cell apoptosis. RBL cells were incubated in a CO_2 incubator (Thermo Scientific Forma Series II Water Jacket, Thermo Fisher Scientific Inc., Waltham, MA, USA).

Construction of a CD63-EGFP prokaryotic expression system. The complete coding sequence of mouse CD63 (NM_001042580) was retrieved from the NCBI GenBank database and amplified from mouse liver cDNA. The primers employed were: mCD63, 5'-cgc gtc gac atg gcg gtg gaa gga g-3' (forward) and 5'-gcg gga tcc cat tac ttc ata gcc ac-3' (reverse). The mCD63 PCR products and apEGFP-N1 vector were digested with BamHI and SalI, and the resulting fragments purified and ligated with T4 DNA ligase. The ligated product was transformed into DH5a competent cells and an isolated positive clone named pEGFP-N1-mCD63.

Transfection of Plasmid DNAs. One day before transfection, plates were prepared containing 2×10^5 RBL mast cells in 500 μL of growth medium without antibiotics such that the cells were 90–95% confluent at the time of transfection. The

ratio of cationic carrier/DNA was optimized based on the manufacturer's instructions. Briefly, 4μg DNA were diluted in 50μL Opti-MEM® I Reduced Serum Medium without serum (Gibco® Invitrogen, Life Technologies) and mixed gently. 10μL of Lipofectamine®2000 (Invitrogen, Life Technologies, Grand Island, NY, USA) were mixed gently before use, and added to 50 μl of Opti-MEM® I Medium. After a 5 minutes incubation at room temperature, diluted DNA were combined with the diluted Lipofectamine® 2000（total volume = 100 μl）and incubated for 20 minutes at room temperature.Then,the mixture were added to RBL cell plates. After 4 h for DNA transfection, the transfection medium was replaced with fresh culture medium and these cells with CD63-GFP plasmid DNA cultured in 35 mm dishes for a few days. Then, stable transfectants were obtained by selection with the antibiotic G418 (Gibco® Invitrogen) and a stable transfectant RBL cell line with EGFP selected.

Western Blot Analysis for CD63-EGFP expression. To each tube of harvested cells (in 20 μL) 180 μL of RIPA lysis buffer containing PMSF was added and incubated for 10 min at room temperature (RT). Equal amounts of proteins were separated using 10% SDS-PAGE after boiling for 5 min in 4× loading buffer and transferred onto Immobilon-P membranes (Millipore Corp., Billerica, MA, USA). The membranes were then incubated with anti-EGFP antibody and a second antibody IgG-HRP (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunoreactivity was detected using the ECL detection system (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). The target protein bands were quantified by scanning densitometry using ImageJ processing software and normalized to the signal intensity of GAPDH.

In vitro cell proliferation and apoptosis assay. RBL cells seeded on a

96-well plate at 2×10^5 cells/well were infected with CD63-EGFP using the procedures described above or received only changes with fresh media, as controls. At 1, 2, or 3 days postinfection, SRB assays were performed as described by Cung at al. [28] In brief, cells were fixed at 4 °C and in 3.3% (w/v) trichloroacetic acid for 1 h without removing the culture media. After removal of the fixation solution by tapping until the plates were completely dry, each well of cells was incubated with 100 μL of 0.057% (w/v) SRB at RT for 30 min. The wells were then rinsed 4 times with 1% (v/v) aqueous acetic acid, dried, and the stained cells were dissolved in 200 μL of 10 mM Tris base (pH 10.5) with shaking for 5 min. Finally, the 510 nm absorbance was measured on a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).The influence of EGFP gene transfer on cell survival was examined by evaluating apoptosis via fluorescence-activated cell sorting analysis including an Annexin-V-EGFP Apoptosis Detection Kit (Nanjing KeyGen Biotech Co., Ltd., Shanghai, China). Briefly, RBL cells on 12-well plates were harvested by trypsinization 3 d after infection with CD63-EGFP plasmid. Following neutralization by washing with culture media containing FBS and phosphate buffered saline, the cells were resuspended in 100 μL of annexin-V binding buffer and stained in the dark with 4 μL annexin-V/EGFP (an apoptosis marker) and 4 μL propidium iodide (PI, a necrosis marker) or 15 min at room temperature as a control. After adding 400 μL of annexin-V binding buffer, 10,000 cells per sample were analyzed by flow cytometry and CellQuest software. Fluorescent signals from EGFP and PI were distinguished by 3 color detectors.

Parvalbumin purification. The PV from crucian carp, bream and silver carp was extracted as described previously. [29] Briefly, 2 g white muscle of each

sample was extracted with 10 mL 20 mM Tris-HCl (pH7.5), using a homogenizer (NS1001 L2K, Niro Soavi, Italy). Supernatants were obtained as PV crude extracts after centrifugation at 8000 g for 30 min. To achieve a clear solution for test, crude extracts were purified by taking advantage of their thermostability, heating at 70 ℃ for 5min, and then immediately cooled in ice water followed by centrifugation at 40000 g for 30 min. supernatants composed mainly by PV were analyzed by SDS-PAGE. Then the extract solutions were subjected into anti-PV antibody pre-sensitized RBL cells which were monitored by CLS microscope for fluorescence analysis.

RBL cell sensor assay. RBL-2H3 cells were cultured in a flask in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin (100 $\mu g \cdot mL^{-1}$), and streptomycin (100 $\mu g \cdot mL^{-1}$) at 37 ℃ in a humidified atmosphere containing 5% CO_2. At the growth retardation stage after 3 d, cells reached to the logarithmic growth phase after which they were plated onto 35-mm glass-bottomed dishes (2×10^5 cells/dish) to form a monolayer overnight. Cells were then washed with Tyrode's buffer (Maishi BioTech, Suzhou, China) and presensitized by incubation with Tyrode's buffer containing 10 mg/mL antibodies and 2.5 mM probenecid at 37℃ for 30 min. The resulting cells were then washed and maintained in Tyrode's buffer. A volume of 50 μL of serially diluted samples containing DNP–BSA or fish PV antigen, were prepared in Tyrode's buffer and subjected to the prepared RBL sensor cells. The cell responses were monitored using an LSM 710 CLS microscope (Carl Zeiss Microscopy GmbH). Time-lapse sequences of RBL cells, recorded every 30 s, were collected and the total fluorescence intensity for each image calculated using Scion Image software (Scion Corp., USA). Cells not exposed to DNP-BSA (or fish PV

antigen) served as controls.

β-Hexosaminidase assay for measurements of cell degranulation. A β-hexosaminidase assay was performed to validate the result of fluorescence analysis by IgE-mediated allergic response. [30] Cells growing in 96-well plates were washed, incubated in Tyrode's buffer, and stimulated with medium containing different allergen concentrations. After 1 h, the supernatant was removed and the cell monolayer lysed in Tyrode's buffer containing 0.5% TX-100 to obtain the total cellular β-hexosaminidase activity. This activity was measured in both supernatant and lysates by adding 50 μL of substrate p-nitrophenyl-N-acetyl-β-glucopyranoside (1 mg/mL) to each well. After incubation at 37 °C for 1 h, the reaction was quenched by addition of 150 μL of 0.4 M glycine, pH 10.7, and the 405 nm absorbance read in an ELISA reader (BioTek Instruments, Inc.). The ratio of β-hexosaminidase release was calculated using the following formula: β-hexosaminidase release (%) = 100 × [(supernatant/blank supernatant)/(supernatant/blank supernatant) + (total cell lysate/blank total cell lysate)]. Cells incubated in medium without allergen served as blanks.

■ RESULTS AND DISCUSSION

Working principles of RBL cell sensors. Scheme 1 illustrates the working principle by which an RBL cell sensor recognizes and quantifies target antigen. In this study, we fused EGFP to mouse CD63, in which CD63's C-terminus was conjugated with EGFP to. Then we introduced the fusion plasmid CD63-EGFP into RBL cells through a highly efficient, lipid-mediated, DNA-transfection procedure.

DNA-lipid complexes were fused with the membrane by endocytosis, and the fluorescent protein gene was diffused throughout the intracellular membranes, thus entering the nucleus. Next, RBL cells were selected with the antibiotic G418 to obtained stable transfectant cells. Results show that CD63 expression on cell surfaces increased, along with the successive expression of fluorescent protein upon antibody activation in RBL mast cells pre-sensitized with allergen antigen. (See Scheme 1. right, enlarged drawing). In this way, we monitored cell responses and quantified the allergen antigen through intracellular fluorescence changes observed under a CLS microscope.

Successful construction and expression of CD63-EGFP chimera proteins. We amplified the CD63 gene from cDNA of mouse liver tissue with oligonucleotides so that SalI and BamHI restriction sites were introduced at the 5' and 3' ends, respectively. The PCR products and pEGFP-N1 plasmids were also double-digested with BamHI and SalI, ligated with T4 DNA ligase, and transfected into DH5a competent cells (Figure 1A). The recombinant plasmids were double-digested with BamHI and SalI, and the positive clone was named pEGFP-N1-mCD63 (Figure 1C). The anti-EGFP antibody was used to detect CD63-EGFP fusion protein expression. One major band, ~46 kDa (the CD63 and EGFP fusion protein), was observed (Figure 1B), which was in good agreement with the sum of the molecular sizes of CD63 and EGFP. The RBL cells infected with CD63-EGFP showed strong fluorescence signals from EGFP expression at day 3 after antigen stimulation, indicating a high transfection efficiency of about (83.27±1.45)%

249 through fluorescent observation and flow analysis (Supplementary material, Figure
250 S1A).

251 SRB-based proliferation assays were performed to investigate potential adverse
252 effects during the transfection process. Results show no difference in RBL cell
253 numbers at 1, 2, and 3 days between the CD63-EGFP infected and control groups
254 (Figure S1B). Flow cytometric analyses were conducted at day 3 for the CD63-EGFP
255 infected and control groups, using annexin V-EGFP and PI as markers for apoptosis
256 and necrosis, respectively. Results show similar low rates of early apoptosis (0.86 ±
257 0.21 vs. 0.72 ± 0.18%, respectively), and necrosis (0.17 ±0.13 vs. 0.23 ± 0.15%,
258 respectively; Figure S1C).

259 **Quantification of fish allergen PV by RBL cell sensors.** Naive RBL
260 cells cannot recognize any antigens. They can only recognize antigens when
261 anti-antigen *Ig*E antibodies bind tightly to the high-affinity receptors (FcεRI) on their
262 cell surfaces. This binding allows them to be stimulated by crosslinking with protein
263 allergens to initiate a sequence of biochemical events, including cellular degranulation
264 and release of inflammatory molecules. [31] We verified the utilization of cell sensors to
265 recognize allergen antigen recognition with DNP-BSA, a multivalent model antigen,
266 to stimulate RBL cells presensitized with anti-DNP *Ig*E.

267 We also used time lapse imaging of cell responses after stimulation with
268 DNP-BSA (100 ng/ml) and measured fluorescence intensity changes over time to
269 determine the amplitude and speed of cell responses. Results show that both declined
270 with decreased DNP-BSA concentration (Figure 2A and 2B, respectively). These

results accord with previous reports. They indicate the presence of target antigens and demonstrate that RBL cell sensor responses can be monitored in real-time with CLSM. [19]

Next, fish PV standards were applied to evaluate the feasibility of RBL cell sensors. We prepared cell sensors using pre-sensitized RBL cells with anti-fish PV antibodies. We monitored the resulting cell fluorescence changes with CLSM in real-time. Time-lapse images of RBL cell sensors responding to fish PV (100 ng/mL) reveal that activating mast cells with antigens led to a rapid and robust increase in cell fluorescence intensity (Figure 3A). We also measured the sensors' response to different PV concentrations in terms of the fluorescence intensity vs. time (Figure 3B). PV samples were added after a stable fluorescence signal was obtained. At low PV concentrations (1–100 ng/mL), from the time of addition (at 0 s), RBL cells showed a slow increase in fluorescence intensity. This indicates the initiation of CD63-EGFP chimera protein expression on cytoplasmic granular and plasma membranes. This, in turn, created a nonuniform distribution of fluorescence on the cell surface and in the cytoplasm, leading to a lower fluorescence intensity.

At high PV concentrations, cell fluorescence intensity rose rapidly, reaching a plateau of sustained saturation within 60 s after stimulation. Further investigation of these cells' allergic responses reveals that high activation of mast cells induced fusion of cytoplasmic granular membranes with plasma membranes. This resulted in the degranulation of secretory vesicles and release of inflammatory molecules, such as histamine and β-hexosaminidase. [32] We also examined fluorescence changes on the

surface of single cells and the result accorded with previous results (Suppl. Figure S2).

A histogram of the linear relationship between the fish PV concentrations and fluorescence intensity clearly show that fluorescence signals follow the same trend as that of DNP-BSA activated cells (Figure 4A). Fluorescence intensity was proportional to PV, ranging from 1 to 100 ng/mL. A β-hexosaminidase assay, which was performed to confirm the fluorescence analysis results (Figure 4A, insert). This indicates that the β-hexosaminidase release rate increased gradually at low PV concentrations, while it grew rapidly at high concentrations. These data agreed well with the dose-response curve obtained from fluorescence measurements.

At both in low and high PV concentrations, fluorescence intensities exhibited good linear relationships with fish PV concentrations, with a correlation coefficient of 0.992 and 0.991(low and high PV concentrations, Figure 4B and 4C, respectively). The limit of detection (LOD) calculated from Figure 4B was 0. 35 ng/mL, according to the formula: LOD = $3s/m$, where s represents the blank sample standard deviation (n=3) and m represents the slope of related PV calibration curve. These measurements were performed in triplicate to assess the reproducibility and precision of freshly fabricated cell sensors. The relative standard deviation (RSD) of the detection results were all <5%, showing acceptable performance.

Real sample measurements. To assess the utility of these sensors for rapidly recognizing target allergens from the complex food matrix, we used crucian carp, bream and silver carp extra to test the performance of this sensor. As shown in

Figure 5A, purified PV was applied to the gel and SDS-PAGE was performed. There was a major protein band at 12 kD which was identified by Coomassie blue staining (Figure 5A arrow). This indicated substantial purification on PV. Then the activation of cell sensors with 3 kinds of fish extracts which also led to a rapid and robust increase in cell fluorescence intensity (Figure 5B). The concentrations and contents of PV in 3 fish samples calculated from the standard curve of the fluorescence intensity vs. PV standards in Figure 4B were shown in Table 1. The results obtained using the RBL sensors were similar to those reported methods, which confirms that this biosensor can be applied effectively for the detection and prediction in real allergen samples.

Investigation of RBL cell sensor specificity and reproducibility. We also assessed the effectiveness of this new sensor for recognition of fish PV by challenging RBL sensor cells that were pre-sensitized with anti-fish PV antibodies, fish PV, shrimp allergen Pen a 1, and peanut allergen Ara h 1 (prepared in-house). Nonspecific interactions between cells and antigens were excluded by challenging naive, unsensitized RBL sensor cells in parallel with pre-sensitized cells. Shrimp allergen Pen a 1 or peanut allergen Ara h 1 produced few observable responses (Suppl. Figure S3). Therefore, it can be assumed that contamination with shrimp and peanut allergens would not influence sensor recognition of fish PV in heterogeneous mixed-food samples containing various allergens.

To demonstrate the reproducibility of this cell-based sensor, measurements were performed in triplicate with same prepared RBL cells to assess reproducibility and

337 precision (SD=0.446±0.04). Moreover, RBL cells were repeatedly activated by
338 anti-PV IgE and PV antigens within a 24-hour interval. Results of fluorescence
339 analysis show that reactivated RBL cells in response to PV levels retained its 80%
340 original value (seen in Suppl. Figure S6) which well illustrates the reproducible
341 characteristics of the sensor.
342

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Figure captions

Scheme 1. A schematic illustration of the working principle of the RBL cell sensor. Fusion plasmid CD63-EGFP was introduced into RBL cells to obtained stable transfectant fluorescent RBL cells. When allergen antigen bound to the antibodies pre-sensitized mast cells, cytoplasmic granules would be fused with the plasma membrane and initiate the expression of CD63 and fluorescent protein on the cell surface. Therefore allergen antigen would be detected by the elevation of intracellular fluorescence emitted by EGFP expression in RBL cell.

Figure 1. Construction of the pEGFP-N1-mCD63 vector and expression of CD63-EGFP protein (A) pEGFP-N1-mCD63 plasmid double digested results. M represents DNA marker (250, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 45000, 5000, 6000, and 7000) bp; 1 represents pEGF P-N1-mCD63 plasmid double digested with Bam HI and Sal I. (B) Western blotting of lysates prepared from RBL cells at 24 and 48 h after transfection with pEGFP-N1-mCD63 vector with anti-EGFP antibodies. M: protein marker; 1: Negative control (Non-transfection Cell); 2: CD63-EGFP fusion protein; 3: Positive control (Cell transfected with pEGFP-N1). (C) Map of pEGFP-N1-CD63 plasmid. Mouse CD63 gene (not including the stop codon) was introduced at the Sal I and Bam HI restriction sites of pEGFP-N1 (Clontech, PT3027-5) vector's MCS region.

Figure 2. Fluorescence intensity changes of mast cells exposed to various concentrations of DNP-BSA. (A) Time-dependent images of cell fluorescence intensity responses. 100 ng/ml of DNP-BSA was employed to stimulate anti-DNP IgE pre-sensitized RBL cells. The images were recorded every five seconds and displayed every thirty seconds. (B) Quantification of fluorescence intensity in RBL cells after sensitization with tenfold serial dilutions of DNP-BSA.

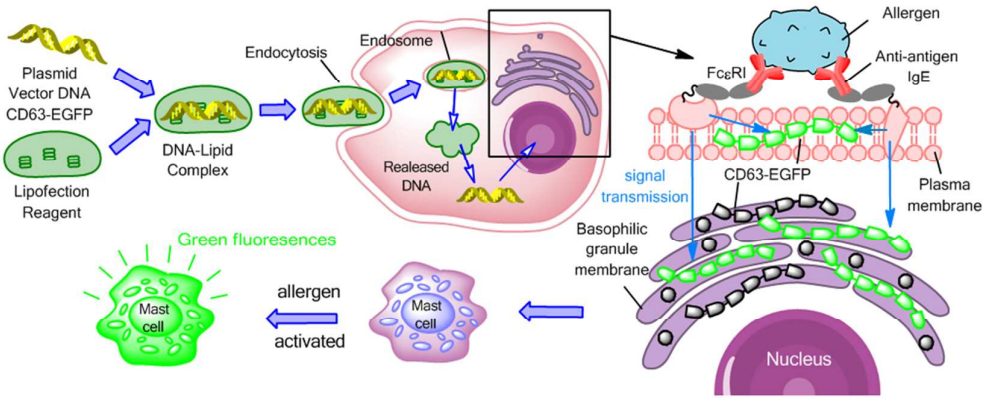
Figure 3. Fluorescence intensity changes of RBL cells with different concentrations of fish Parvalbumin (A) Time-dependent images of cell fluorescence intensity variation. 100ng/mL of Parvalbumin was used to stimulate RBL cells that pre-sensitized with anti-Parvalbumin antibodies. The images were recorded at an interval of five seconds and exhibited every thirty seconds. (B) Quantification of fluorescence intensity in RBL cells exposed to 10 different concentrations of

Parvalbumin(from a to k: 0ng/mL , 1ng/mL, 2.5ng/mL, 5ng/mL, l0ng/mL, 20ng/mL, 40ng/mL, 60ng/mlL, 80ng/mL, 100ng/mL).

Figure 4. Quantification of fish allergen parvalbumin by RBL-2H3 cell-based sencor: (A) fluorescence intensity spectroscopy for RBL-2H3 cells treated with different doses of fish allergen parvalbumin: 1ng/mL, 2.5ng/mL, 5ng/mL, 7.5ng/mL, 10ng/mL, 20ng/mL, 40ng/mL, 60ng/mL, 80ng/mL and 100ng/mL. insert : β-hexosaminidase assay for confirmation of cell activated by fish allergen parvalbumin (B). Linear regression of fluoresence intensity versus low concentrations of parvalbumin(1ng/mL to 10ng/mL). (C) Linear regression of fluoresence intensity versus high concentrations of parvalbumin(10ng/mL to 100ng/mL).Data represent mean±SE of three different experiments under similar condition.

Figure 5. A:SDS-PAGE analysis for parvalbumin from 3 kinds of different fish purification. M: protein marker (97, 66, 44, 29, 20, 14kDa); 1: crucian carp;2: silver carp;3: bream .B:Quantification of 3 kinds of fish parvalbumin by RBL-2H3 cell-based sensor: (a) crucian carp (b) silver carp (c) bream

Table 1. The concentration and content of parvalbumin in 3 kinds of fish



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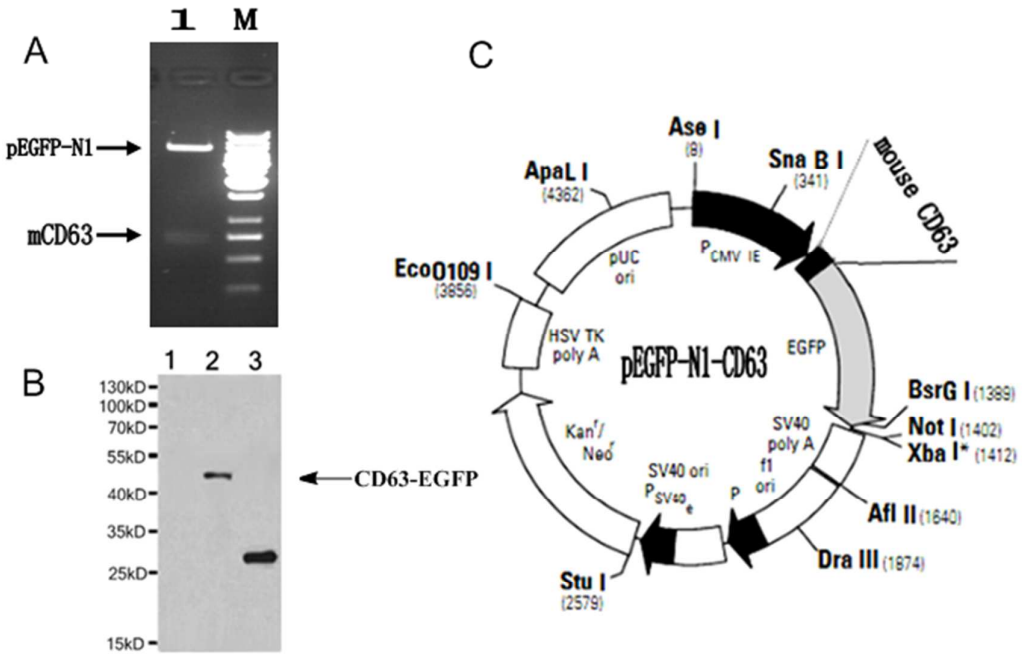

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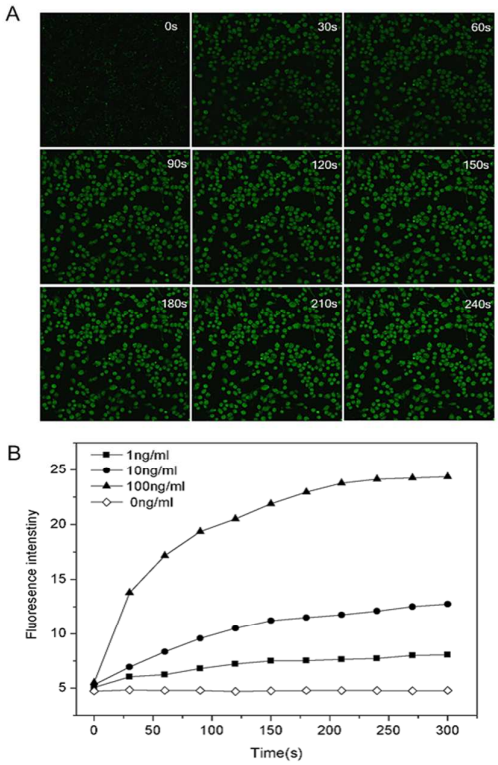

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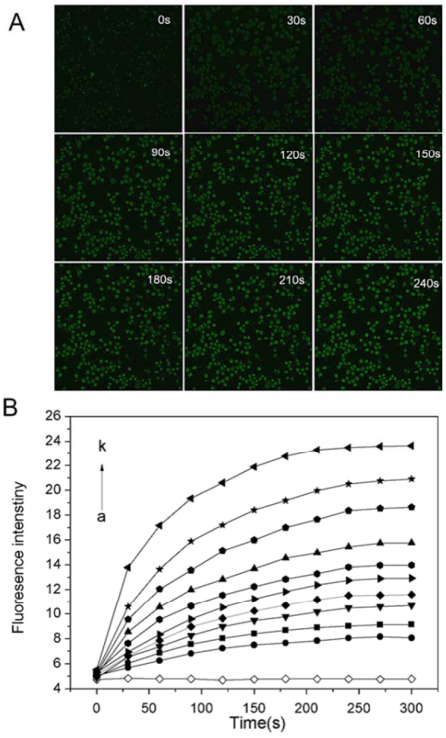

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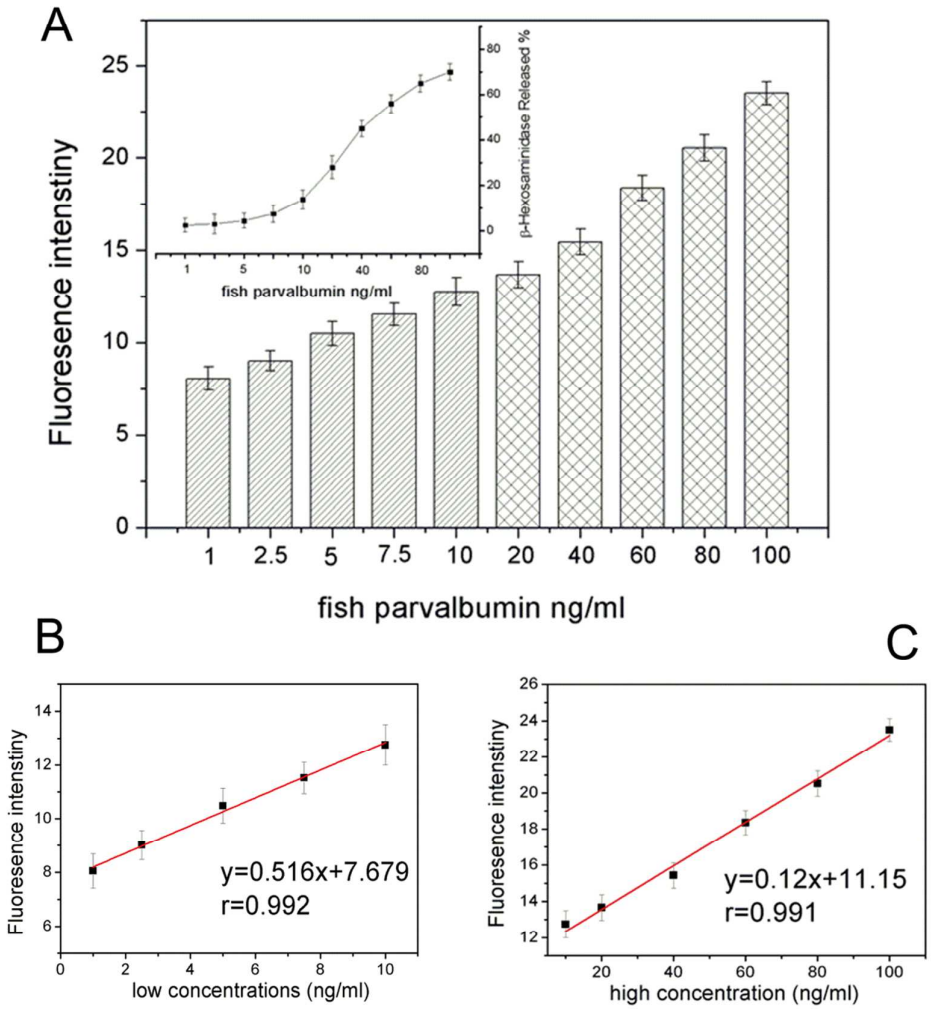

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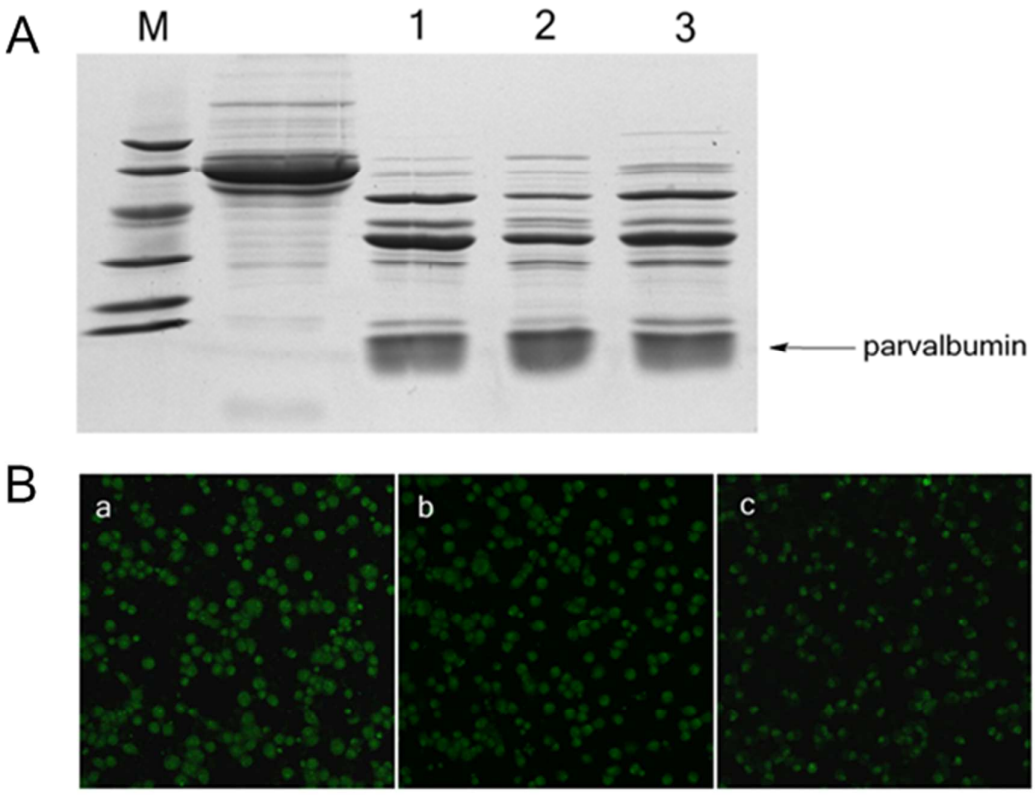

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552 **Table1.** The concentration and content of parvalbumin in 3 kinds of fish

Sample	Crucian carp	Silver carp	bream
Fluorescence intensity （mean±SD）	18.84±0.08	14.88±0.14	13.68±0.16
PV concentration(ng/ml±SD)	64.08±0.22	31.08±0.35	21.08±0.33
PV content (mg/g±SD)	1.92±0.23	0.93±0.28	0.63±0.26

553 SD,standard deviation (n = 5).

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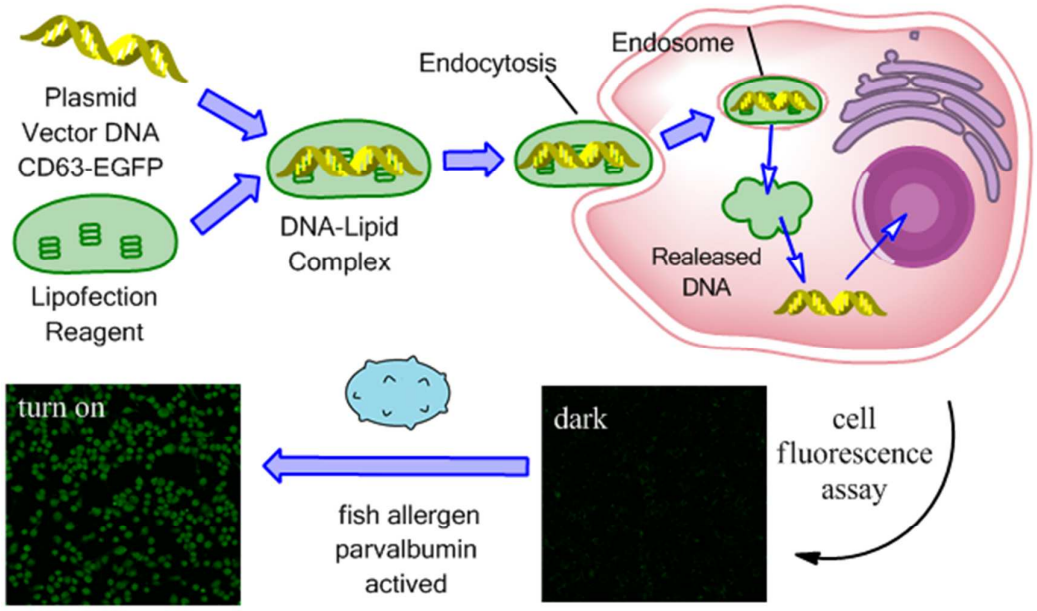
555 **Toc Graphic**



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