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Label-free liquid crystal biosensor for cecropin B detection

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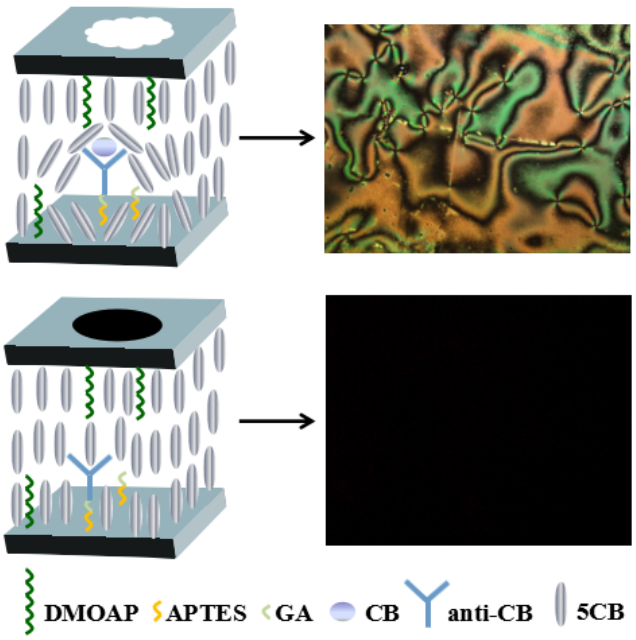
ABSTRACT

A label-free liquid crystal (LC) biosensor based on orientation changes of LC molecules was reported for the detection of cecropin B. The homeotropic-to-tilted alignment transition of LC molecules, induced by the specific binding event between cecropin B and anti-cecropin B antibody immobilized via glutaradehyde, could result in obvious change of the optical appearance from a dark to a bright response and as a result, the detection limit of cecropin B was as low as 50 ng/mL. The average gray-scale intensties (GIs) of optical appearances were calculated to quantitatively analyse cecropin B concentrations. This study offers a simple, highly sensitive and specific, lable-free method for cecropin B detection.

Graphical Abstract



KEYWORDS: Liquid crystal; Biosensor; Cecropin B; Anti-cecropin B antibody

1. Introduction

Liquid crystals (LCs) are anisotropic materials with liquid like flow properties as well as solid like optical properties[1]. The alignment of LCs is extraordinarily sensitive to physical and chemical properties of a bounding interface due to the low anchoring energy[2,3]. Therefore, LC-based sensors can transform microcosmic orientation transiation induced by chemical and biological binding events on surface into macroscopic optical signals. In 1998, Abbott and co-workers[4] firstly used LCs as "sensitive element" to amplify and transduce receptor-mediated binding of proteins at surfaces into optical outputs, obseved by naked eyes under the crossed polarizers for avidin (Av) detection. This novel liquid crystal biosensing approach permited a label-free, highly sensitive and specific, low-cost, simple and fast detection for analytes. In the past nearly two decades, considerable research efforts have been devoted to LC-based sensors for the detections such as tyrosine[5,6], cholesterol[7-9], glucose[10-12], antigens[13], proteins[14-16], nucleins[17,18], heavy-metal ions[19] and so on. Also, LC biosensing technology has been applied to image molecular interactions between cationic antimicrobial peptides(PGLa) and lipid membrane[20].

Cecropin B (CB) is the first inducible antimicrobial peptides found in 1980 from *hyalophora cecropia*[21]. Antimicrobial peptide exhibits a broad spectrum of antimicrobial activities against both gram-negative[22] and gram-positive bacteria[23], fungal[24], cancer cell[25] and virus[26] so that it has great potential application value in the fields of animal and plant disease resistance gene engineering[27,28], plant breeding[29], biological feed additive[30]. In recent years, different analytical methods were developed for polypeptides detection, for instance, liquid chromatography-mass spectrometry[31], capillary electrophoresis[32] and immunoassay[33]. Most of these methods could sensitively and accurately detect polypeptides, however, it must also be mentioned that they need complicated pretreatment or derivatization, fluorescence labels for the samples and expensive laboratory equipment. LC biosensors are portable, low-cost and avoid complex fluorescence labels for biomolecules during the detection procedure, superior to traditional analytical approaches, making them well suited for analytes detection. Despite, Hu et al[20] have studied membrane disruption or

permeabilization caused by antimicrobial agents using LCs, confirming that there existed electrostatic interactions between the cationic antimicrobial (PGLa) and the negatively charged phospholipid. To date, very little report is available for low-cost, lable-free, highly sensitive and specific cecropin B dection by liquid crystal biosensor, which is of great importance.

In this study, a simple LC biosensing strategy for the highly sensitive detection of cecropin B was proposed through disrupting the LCs orientation based on specific binding event beween cecropin B and anti-cecropin B antibody as shown in Figure. 1. Firstly, the surface of a plain glass slide substrate was decorated with the mixed self-assembled monolayers of *N*,*N*-dimethyl-*N*-octadecyl(3-aminopropyl) trimethoxysilyl chloride (DMOAP) and (3-aminopropyl) trimethoxysilane (APTES) with both long and short alkane thiols (Fig. 1b), which can not only induce LCs vertical alignment to obtain a black background but also provide plenty of amino groups reacting with glutaraldehyde (GA) to immobilize anti-cecropin B antibody. Subsequently, anti-cecropin B antibody was immobilized on the chemically functionalized substrate through the coupling reaction between the amino groups and aldehyde groups of GA (Fig. 1c) , and a black background could still be observed under the crossed polarized light in the absence of cecropin B, after combining with DMOAP-coated glass slide to fabricate LC cell filled with 5CB (Fig. 1f). In the presence of cecroin B, colorful optical image could be observed under the crossed polarized light via the steric disrupting effect on the LCs alignment, casued by the specific binding event between cecropin B and anti-cecropin B antibody (Fig. 1e). Moreover, we investigated the correlations between average gray-scale intensities of optical images of LC cells and cecropin B concentrations to quantitatively detect cecropin B. In comparison with traditional analytical approaches, lable-free, highly sensitive and specific cecropin B detection could be achieved by LC biosensor.

2. Material and methods

2.1. Reagents and materials

Premium grade glass slides were obtained from Jiangsu Feizhou Plastic Co., Ltd (Jiangsu, China). *N*,*N*-dimethyl-*N*-octadecyl (3-aminopropyl)trimethoxysilyl chloride (DMOAP, 42% w/w), (3-aminopropyl) triethoxysi-lane (APTES, 99%), cecropin B (CB, ≥97%) were purchased from Sigma-Aldrich (St. Louis, USA). Anti-cecropin B antibody (anti-CB), human β defensin-2 and human β defensin-3 were obtained from Abcam (Cambridge, UK). Bovine serum albumin (BSA), bovine hemoglobin (BHb) were purchased from BD (New Jersey, USA). Nematic liquid crystal

4-cyano-4-pentylbiphenyl (5CB) was obtained from Instec (Colorado, USA). Glutaraldehyde (GA) and other reagents were all of analytical reagent grade. All the reagents were used as received without further purification. Ultrapure water (18.2 MΩ*cm) was used throughout all this report.

2.2. Cleaning of substrates

The glass slides cut into 2 cm × 2 cm square were cleaned with freshly prepared piranha solution (70% H_2SO_4, 30% H_2O_2) at 80 ℃ for 2 h to remove all organic contaminants, and then, rinsed with copious amounts of ultrapure water, dried under a stream of nitrogen, and heated in a 110 ℃ oven at least 3 h before use.

2.3. DMOAP-decoration

The cleaned glass slides were immersed in an aqueous solution containing 0.2% (v/v) DMOAP at room temperature for 30 min, and then rinsed with copious amounts of ultrapure water, dried under a stream of nitrogen and heated in a 110 ℃ oven for 1 h.

2.4. APTES/DMOAP-decoration

The cleaned glass slides were immersed in ethanol solution containing 5% (v/v) APTES and 1% (v/v) DMOAP at 80℃ for 2 h, rinsed with ethanol to remove any physically absorbed APTES/DMOAP, and then rinsed with ultrapure water, dried under a stream of nitrogen, heated at 110 ℃ for 1 h. Subsequently, the glass slides were immersed in a 2.5% (v/v) GA solution for 1h, rinsed with ultrapure water, dried under a stream of nitrogen.

2.5. Anti-cecrpin B antibody immobilization

Firstly, anti-cecropin B antibody was dissolved in a 10 mM PBS buffer (pH = 7.4, 137 mM NaCl, 2.7 mM KCl). Subsequently, the prepared solution was dipped on GA-activated glass slides at 37 ℃ for 1 h, and then rinsed with 10 mM PBS buffer (pH = 7.4, 137 mM NaCl, 2.7 mM KCl) and ultrapure water, finally, dried with nitrogen flow.

2.6. Specific binding event between cecropin B and anti-cecropin B antibody

Different concentrations of cecropin B solutions were dipped on glass slides immobilized with anti-cecropin B antibody at 37 ℃ for 2 h, then rinsed with 10 mM PBS buffer (pH = 7.4, 137 mM NaCl, 2.7 mM KCl) and ultrapure water to remove nonspecific adsorbed molecules, lastly, dried under a stream of nitrogen.

2.7. LC cells

The LC cells were constructed through spacing upper glass slides decorated with DMOAP and lower glass slides decorated with the mixed APTES/DMOAP face to face with thin polyester film of Mylar (19 μm), and then held together with small binder clips. The LC cells were placed at 37 ℃ for 10 min. 5CB is heated at 40℃ for 10 min in a constant temperature chamber from liquid crystalline phase to isotropic phase, and then a small amount of 5CB is spontaneously injected into the LC cells by the capillary action. Subsequently, the LC cells were slowly cooled to ~28 ℃, examined with a polarized light microscope (XPL3230, Shanghai optical instrument No. 1 Factory, China) under the crossed polarizers. The optical appearances were captured by a digital camera mounted on the microscope. The gray-scale intensities (GIs) of the optical appearances were calculated using Adobe Photoshop CS5 software.

2.8. Atomic force microscope (AFM) measurements

The morphology of substrate modified with the mixed APTES/DMOAP self-assembled monolayer was characterized using the atomic force microscope (SPI3800N/SPA400, Seiko, Japan). The AFM images were obtained in the contact mode with a scan rate of 1.2 Hz and scanning scope of 2 μm×2 μm.

2.9. Water contact angle measurements

The water contact angles of substrates modified with different self-assembled monolayers were measured using the video optical contact angle measurement (OCA20, Dataphysics, Germany). In a typical run, drops of water (5 μL) were placed on the sample surfaces, allowed to stand for 2 min, and images were captured by OCA20 software provided by the manufacturer.

3. Results and discussions

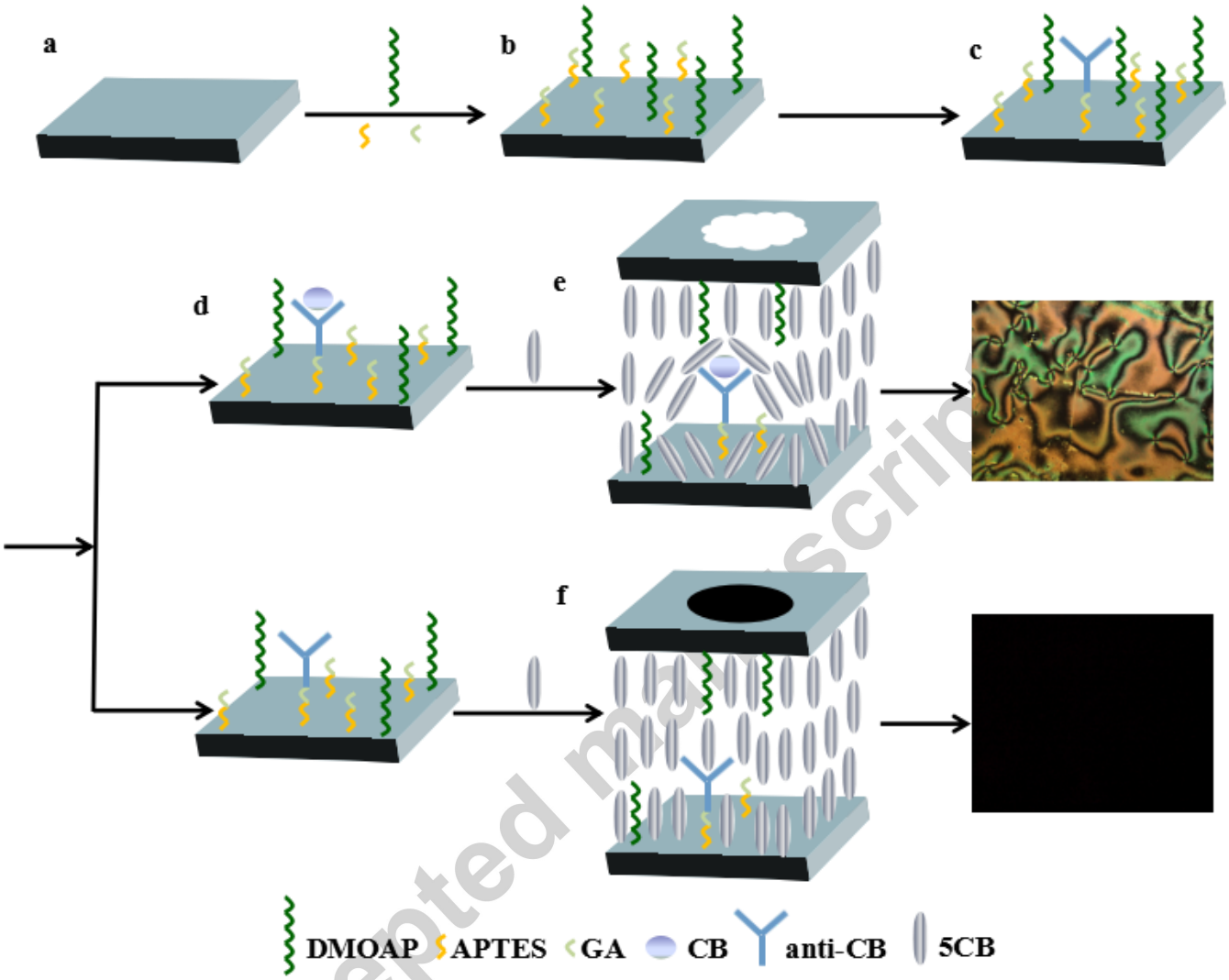


Fig. 1. Illustration diagram of detection strategy and preparation steps for cecropin B-based liquid crystal biosensor. (a) Cleaned glass slide; (b) self-assembled monolayer of APTES/DMOAP functionalized with GA; (c) immobilization of anti-cecropin B antibody; (d) cecropin B specific binding to the anti-cecropin B antibody; (e) orientation disturbtion of 5CB in the cells with cecropin B; (f) homeotropic orientation of 5CB in the cells without cecropin B.

3.1. Modification and characterization of substrate

It has been investigated that the effective birefringence (Δn_{eff}), which is relative to the color and brightness of LC film, varied with alignment layers[34]. Accordingly, the surface modification of substrate is critical for the detection of biomolecules by LC biosensor, in the current process of LC biosensor construction. In this work, the mixed self-assembled monolayer of APTES/DMOAP was

employed to build sensitive surface to induce vertical alignment of 5CB for reducing the background signals. As shown in Fig. S1a, when APTES/DMOAP ratio was 25:1, numbers of bright spots appeared in the optical image of LC cell fabricated by DMOAP-coated glass slide and the mixed APTES/DMOAP-coated glass slide. And the bright spots decreased with the decrease of the APTES/DMOAP ratio. A uniform dark background could be observed when the APTES/DMOAP ratio reduced to 5:1 in Fig. S1b because higher level of DMOAP can adequately trigger the homeotropic alignment of LC molecules. On the basis of APTES/DMOAP ratio at 5:1, the optical image of LC cell showed a uniform dark background shown in Fig. S1c, as the mixed APTES/DMOAP self-assembled monolayer was activated by 2.5% GA, the other aldehyde group of which would couple with amino group to immobilize anti-cecropin B antibody.

The orientation of LCs close to a substrate surface is not only affected by the surface chemical composition and topographical structure but also affected by the surface energy[17]. An AFM image of the APTES/DMOAP-coated glass slide in Fig. 2 showed the peak-valley topography structure with higher roughness than bare glass slide, attributed to the mixed self-assembled monolayer of APTES/DMOAP with long and short alkyl chains, facilitating vertical insertion of LC molecules without background signals generating. The largest peak-to-valley-height (LPVH) was 14.76 nm and the root-mean-square (rms) roughness (Rq) was 2.714 nm, in particular.

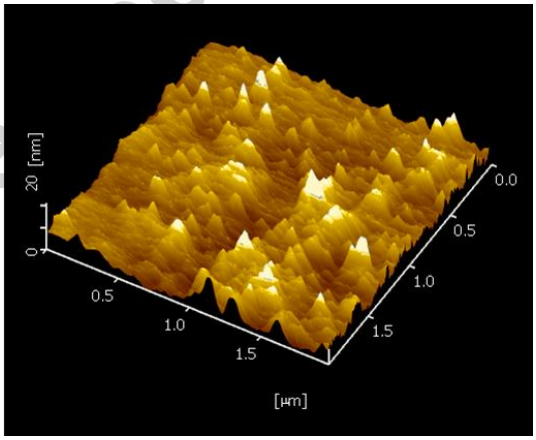


Fig. 2. AFM three-dimensional image of the surface of an mixed APTES/DMOAP self-assembled monolayer at a volume ratio of 5:1 deposited on a glass substrate.

To gain more insight, water contact angle measurements were performed to investigate the influence of surface energy on orientation of LC molecules. As can be seen from Fig. 3a, after cleaned with freshly prepared piranha solution, the water contact angle of the glass slide surface was 18.1° due

to the formation of hydroxyl-riched surface, which was hydrophilic and had low affinity with hydrophobic 5CB molecules[35]. The water contact angle of glass slides surfaces decorated with APTES and DMOAP were 62.5° (Fig. 3b), 87.6° (Fig. 3d), respectively, and it increased with the decrease of APTES/DMOAP ratio, reaching 77.0° eventually when the volume ratio of APTES/DMOAP was 5:1. Under this condition, DMOAP with long alkyl chains could orientate LC molecules perpendicularly to obtain a dark background and APTES provided considerable amino groups to react with aldehyde group of GA , at the same time. Naturally, the second aldehyde group of GA can couple with amino terminated anti-cecropin B antibody. Therefore, the APTES/DMOAP volume ratio of 5:1 should be adopted in the following experiments.

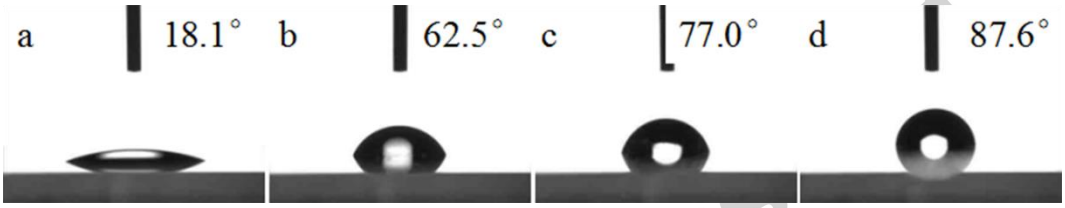


Fig. 3. Water contact angles of different self-assembled monolayers deposited on a glass substrate. Glass slides were treated by (a) piranha solution; (b) APTES; (c) APTES/DMOAP (volume ratio is 5:1); (d) DMOAP.

3.2. Cecropin B detection by LC-biosensor

Considering that the orientation of LC molecules is extremely sensitive to surface topographical structure of substrate, therefore, it is essential to investigate the influence of cecropin B specific bonding to anti-cecropin B antibody on optical images under the crossed polarizers of LC cells. The optical image of LC cell was dark when anti-cecropin B antibody concentration is 1000 ng/mL in the absence of cecropin B shown in Fig. S2, reflecting that the orientations of LC molelules remained homeotropic. As can be seen from Fig. 4, the optical appearances of the LC cells showed a mass of bright spots and obvious interference colors when the concentrations of cecropin B were ≥ 250 ng/mL, indicating that the homeotropic-tilted-planar alignment transition of LC molecules occured on the surface immobilized with 1000 ng/mL anti-cecropin B antibody. The disruption of homeotropic orientation and birefringence behavior of LC molecules resulted from intensive antigen-antibody interactions at higher cecropin B cencentrations. A few bright spots in the optical image under the crossed polarizer of LC cell could be still observed, as the cecropin B cencentration decreased to 50 ng/mL. In contrast, a uniform dark background appeared when cecropin B cencentration was 10 ng/mL, due to that low density cecropin B specific binding to anti-cecropin B antibody is unable to disturb the ordered arrangement of LC

molecules. On the basis of our findings, it can be concluded that the detection limit of of cecropin B was as low as 50 ng/mL.

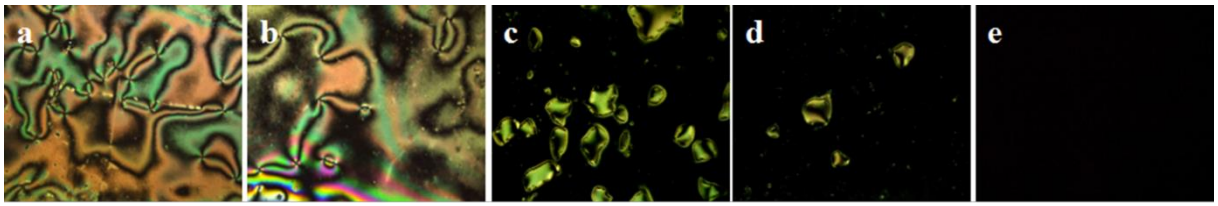


Fig. 4. Optical images the under the crossed polarizers of LC cells in the presence of cecropin B with 5CB sandwiched between DMOAP-coated glass slides and the mixed APTES/DMOAP-coated glass slides assembled with anti-cecropin B antibody. The cecropin B concentrations are (a) 2000, (b) 1000, (c) 250, (d) 50, (d) 10 ng/mL.

To further quantitatively analyse cecropin B concentrations, we tried to investigate the correlations between the average gray-scale intensities (GIs) of optical images under the crossed polarizers of LC cells and the cecropin B concentrations using Adobe Photoshop CS5 software. The GIs value increased with the cecropin B concentration improving in Fig. 5, overall, and the rate of GIs value enhancement was fast at low cecropin B concentrations whereas slow at high cecropin B concentrations. Particularly, the plot of GI values as a function of the cecropin B concentrations between 10-250 ng/mL exhibited linear relationship with a high correlation coefficient (R^2) of 0.9945, suggesting that the quantitative analysis of cecropin B concentrations could be achieved by liquid crystal biosensor below 250 ng/mL. It is worthwhile noted that the proposed LC biosensing approach could sensitively and quantitatively detect cecropin B within a lower concentration range.

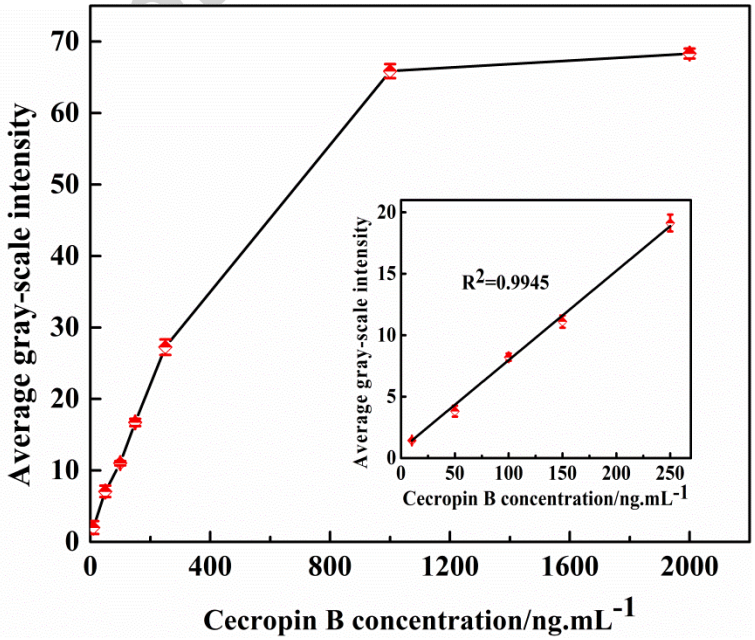


Fig. 5. Correlations between the average gray-cale intensities (GIs) of optical images under the crossed polarizers of LC cells and the cecropin B concentrations. Inset: Linear relationship between the gray-scale intensities and the cecropin B concentrations. The average gray-scale intensity is taken as the average value of the four measurements of a sample for each assay.

3.3. Selectivity of LC-biosensor

For the sake of demonstrating the selectivity of the LC biosensor based on spicific binding event between cecropin B and anti-cecropin B antibody, 2000 ng/mL human β defensin-2, 2000 ng/mL human β defensin-3, 2000 ng/mL bovine hemoglobin (BHb) and 2000 ng/mL bovine serum albumin (BSA) were assembled on the surface of substrate immobilized with anti-cecropin B antibody to fabricate LC cells filled with 5CB by combining with DMOAP-coated glass slides. The optical images were shown in Fig. 6, and it was found that the optical images of human β defensin-2, human β defensin-3, BHb and BSA were still dark under the crossed polarizers with few bright spots, while the optical image of 2000 ng/mL cecropin B showed plenty of bright spots and interference color domains. Since nonspecific peptides and proteins of human β defensin-2, human β defensin-3, BHb and BSA having no specific binding to the anti-cecropin B antibody, could not disturb the ordered alignment of LC molecules, indicating that the LC biosensor based on anti-cecropin B antibody has a good selectivity to cecropin B.

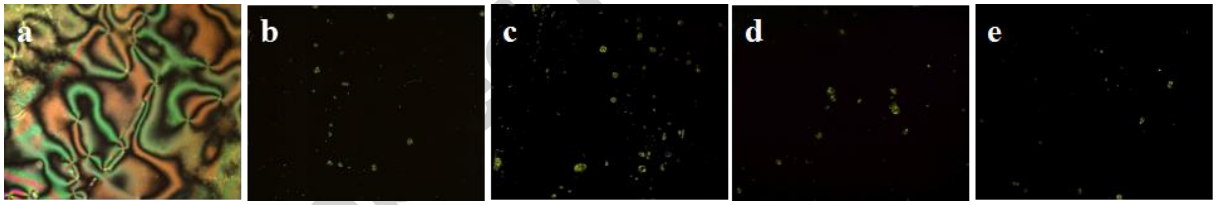


Fig. 6. Selectivity of LC biosensor based on anti-cecropin B antibody immobilized on surface of substrate modified with the mixed APTES/DMOAP self-assembled monolayer. Both of (a) cecropin B; (b) human β defensin-2; (c) human β defensin-3; (d) bovine hemoglobin (BHb); (e) bovine serum albumin (BSA) are at the concentrations of 2000 ng/mL.

Conclusions

In summary, we provided a novel LC biosensing protocol for highly sensitive and specific, lable-free detection of cecropin B based on specific bindning event beween cecropin B and anti-cecropin B antibody, and the detection limit of cecropin B was as low as 50 ng/mL. The anti-cecropin B antibody as the recognition element of the LC biosensor, immobilized on a sensitive substrate via coupling with GA, was able to specifically bind to cecropin B. And this specific bindning event could greatly change surface topographical structure of substrate and induce a

homeotropic-to-tiled orientation transition of LC molecules resulting in strong optical signals observed by naked eyes. Also, quantitative analysis were carried out through average gray-scale intensities of optical images under the crossed polarizers of LC cells. The proposed LC biosensing approach permitted a simple, highly sensitive and specific, lable-free detection for cecropin B and could be potentially applied in other biomolecules detection via various types of binding events.

Acknowledgments

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Highlights

a. A label-free, highly sensitive liquid crystal biosensor for cecropin B detection was proposed.

b. Quantitative detection of cecropin B could be achieved.

c. The proposed LC biosensing strategy toward cecropin B had a good selectivity.