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Specific detection of avidin–biotin binding using liquid crystal droplets

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ABSTRACT

Poly(acrylicacid-b-4-cynobiphenyl-4′-undecylacrylate) (PAA-b-LCP)-functionalized 4-cyano-4′-pentylbiphenyl (5CB) droplets were made by using microfluidic technique. The PAA chains on the 5CB droplets, were biotinylated, and used to specifically detect avidin–biotin binding at the 5CB/aqueous interface. The avidin–biotin binding was characterized by the configurational change (from radial to bipolar) of the 5CB droplets, as observed through a polarized optical microscope. The maximum biotinylation was obtained by injecting a >100 μg/mL biotin aqueous solution, which enabled a limit of detection of 0.5 μg/mL avidin. This droplet biosensor could specifically detect avidin against other proteins such as bovine serum albumin, lysozyme, hemoglobin, and chymotrypsinogen solutions. Avidin detection with $5CB_{PAA\text{-}biotin}$ droplets having high sensitivity, specificity, and stability demonstrates new applications of the functionalized liquid crystal droplets that can detect specific proteins or other analytes through a ligand/receptor model.

1. Introduction

Liquid crystal (LC) droplets are considered a promising platform for sensing applications. LC droplets exhibit a large surface-area-to-volume ratio, which facilitates the reorientation of the LCs at their interfaces. In addition, LC droplets possess several director configurations, which enable them to provide in-depth quantitative information of analytes [1]. LC droplets dispersed in an aqueous solution have recently emerged as simple probes to detect the adsorption and interaction of biological species such as proteins [1,2], lipids [3], endotoxin [4], glucose [5], and urea [6] at the LC/aqueous interface. It is known that the director configurations of the LC droplets, such as radial, preradial, axial, and bipolar, reflect the balance between the elasticity and the surface anchoring of the LCs inside the droplets [7]. The adsorption and interaction of biological species at the LC/aqueous interface may disrupt this balance, inducing a configurational transition of the LC inside the droplets, which can be observed through a polarized optical microscope (POM) [8,9].

Recently, the surface of the aqueous-dispersed LC droplets functionalized by the adsorption of polyelectrolytes (PEs) at the LC/aqueous interface has been utilized in nonspecific biosensors [2,10–14]. The dissociation of the electrolyte groups from PEs induces a charged state, which creates an electric field and changes the configuration of the LC droplet [6]. Several PEs were tested for nonspecific protein detection with LC droplets. For example, poly(acrylicacid-b-4-cyanobiphenyl-4′-oxyundecylacrylate) (PAA-b-LCP) functionalized 4-cyano-4′-pentylbiphenyl (5CB) (a nematic LC at room temperature) droplets made with microfluidics have been used as biosensors for nonspecific protein detection [13]. Another approach for protein detection was the functionalization of LC droplets with surfactants. For example, the pH- and temperature-responsive 5CB droplets functionalized with poly(N-isopropylacrylamide-b-4-cyanobiphenyl-4′-undecaylacrylate) (PNIPAM-b-LCP) and sodium dodecyl sulfate (SDS) have been used for protein detection between the low critical solution temperature and the isoelectric points of tested proteins [15]. However, the specific detection of analytes using 5CB droplets as biosensors through a receptor/ligand model is more highly desirable than nonspecific detection.

Detection of the ligand/receptor binding is the foundation for screening of a specific analyte. Current methods used to detect the ligand/receptor binding generally require an analytical lab apparatus or a bulk assay that involves species labeled with latex beads, enzymes, radioactive isotopes, or fluorophores [16]. The biotin–avidin binding is a well-known receptor/ligand model that is used to amplify and transduce receptor-mediated binding. Avidin is an egg white protein that binds the vitamin biotin with highly

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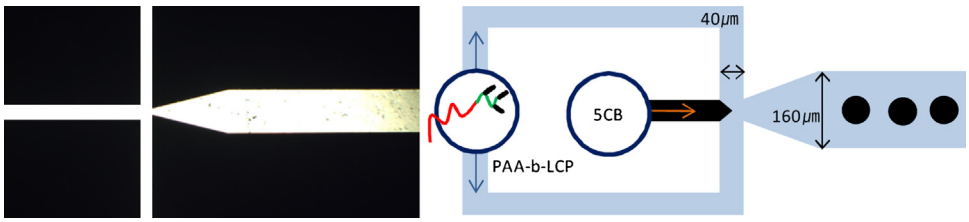


Fig. 1. An optical image (left) and a schematic (right) of the microfluidics channel with dimensions.

specific affinity. The non-covalent avidin–biotin binding provides a general bridge for many diverse applications with an affinity constant of 10^{15} L/mol [17,18], one of the highest affinity constants reported (~10^3 to 10^6 times greater than that for the interaction of other ligands with their specific antibodies) [18–21]. This affinity ensures that once the avidin–biotin complex is formed, it will not be disturbed by a change in pH, the presence of chaotropes, or assay protocols such as multiple washes. Due to the formation of this highly stable complex, the biotin–avidin interaction has become very useful in a wide variety of bioanalytical applications such as affinity, chromatography, and biosensors [17,21,22].

In this study, PAA-b-LCP-functionalized 5CB droplets were generated by a microfluidic technique to demonstrate the selectivity of the LC droplet biosensor by employing the specific receptor on the LC droplet. The PAA chains coated on the 5CB droplets were strongly bound to the LCP block and were biotinylated ($5CB_{PAA\text{-}biotin}$) with covalent coupling. The droplets were tested for the specific detection of avidin at the 5CB/aqueous interface. The specific avidin–biotin complex exhibited a radial-to-bipolar (R-B) orientation change of the 5CB droplets, as observed through a POM. The R-B orientation change of the $5CB_{PAA\text{-}biotin}$ droplets in response to avidin–biotin binding may establish a new platform for the specific detection of proteins and other analytes.

2. Materials and methods

2.1. Materials

5CB (TCI Japan), poly(dimethylsiloxane) (PDMS) kit (Sylgard 184; Dow Corning, USA)containing the prepolymer and a cross-linker, biotin hydrazide, avidin, rhodamine 6G, N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysulfosuccinimide (NHS), bovine serum albumin (BSA), hemoglobin (Hb), chymotrypsinogen (ChTg), and lysozyme (Lyz) were bought from Sigma–Aldrich. Milli-Q water (resistivity higher than 18.2 MU cm) was used in all experiments. Micro-slide glasses (S9213, Matsunami, Japan, 76 mm × 52 mm × 1.3 mm) were cleaned using a hot piranha solution (H_2O_2 (35%): H_2SO_4 (98%) = 1:1 (v/v)) for 30 min, rinsed with water, and dried with nitrogen gas. CAUTION: Piranha solution is extremely corrosive and must be handled carefully. PAA-b-LCP was prepared using the same method reported previously [23]. The molecular weight was PAA(15k)-b-LCP(7k) with a M_w/M_n of 1.19; the number in parenthesis represents the number average molecular weight (M_n), which was calculated from the gel permeation chromatography (GPC) data of poly(tert-butyl acrylate) (PtBA)-b-LCP using 100% conversion.

2.2. Device fabrication

The flow-focusing devices were fabricated by the same method as reported [5]. Briefly, the PDMS was prepared by mixing the prepolymer and the cross-linker thoroughly at the supplier's recommended ratio of 10:1 (w/w). The PDMS was degassed for 40 min in a desiccator to remove the remaining air bubbles. The final mixture was poured on a silicon wafer mold and cured inside an oven at 65 °C for 4 h before removing from the silicon wafer. This patterned piece of PDMS was bonded to a pre-cleaned micro-slide glass using a 46-s oxygen plasma treatment (Femto Science Inc., Korea). The width of the inlet channels, width and length of the orifice, and width and height of the outlet channel were 110, 40, 40, 160, and 40 μm, respectively, and the depth throughout the channel was 100 μm. Fig. 1 shows an optical image and a schematic of the microfluidics channel with dimensions. The channel walls and chip assembly were made hydrophilic by an oxygen plasma treatment. The channel was filled with water until the chip was used.

2.3. $5CB_{PAA}$ droplet formation and its biotinylation

Formation of LC droplets was carried out using the same method previously reported by Khan et al. [24]. Briefly, the liquids were supplied to a microfluidic device via a flexible plastic tubing (Norton, USA, I.D. 0.51 mm, O.D. 1.52 mm) attached to precision syringes (SGE Analytical Science, Australia) operated using digitally controlled syringe pumps (KD Scientific, KDS 100 Series, USA). The flow rates through the microfluidic channels were controlled using two independent syringe pumps. The continuous and dispersed phases were an aqueous PAA-b-LCP (2 mg/mL) solution and 5CB, respectively. The continuous phase was pumped into the two side inlet channels, and the dispersed phase was delivered to the middle channel. Both phases met at the junction, and droplet formation took place when the fluids crossed the neck of the channel. The typical flow rates used for droplet formation were 0.01 and 0.2 mL/h for the dispersed and continuous phases, respectively. The $5CB_{PAA}$ droplets were acquired in a reservoir, and the PAA chains were activated with 0.4 M EDC·HCl and 0.1 M NHS for 1 h. The activated $5CB_{PAA}$ droplets were kept in a biotin hydrazide solution for 12 h at room temperature to obtain biotin-functionalized 5CB droplets ($5CB_{PAA\text{-}biotin}$ droplets). The tested concentrations of the biotin solution (C_bs) were 5, 7.5, 10, 50, 75, and 100 μg/mL.

2.4. Labeling of avidin

The labeling of avidin was carried out following a method reported by Khan et al. with a slight modification [6]. Briefly, avidin was dissolved in phosphate buffered saline (PBS) buffer (pH = 7) in a reaction vial to obtain a 100 μg/mL solution into which the chemical coupling agents, EDC·HCl and NHS, were added and kept for 1 h at 4 °C to activate the carboxyl group in avidin. Subsequently, 1 mg of rhodamine 6G was added and stirred for 12 h at room temperature. A saturated aqueous ammonium sulfate solution (0.5 g/mL) was added dropwise to the mixture. The labeled avidin ($avidin_{rhodamine\ 6G}$) was precipitated and centrifuged twice at 5000 rpm for 20 min. The supernatant was then removed by filtration, and the filtered $avidin_{rhoddamine\ 6G}$ powder was dried under vacuum.

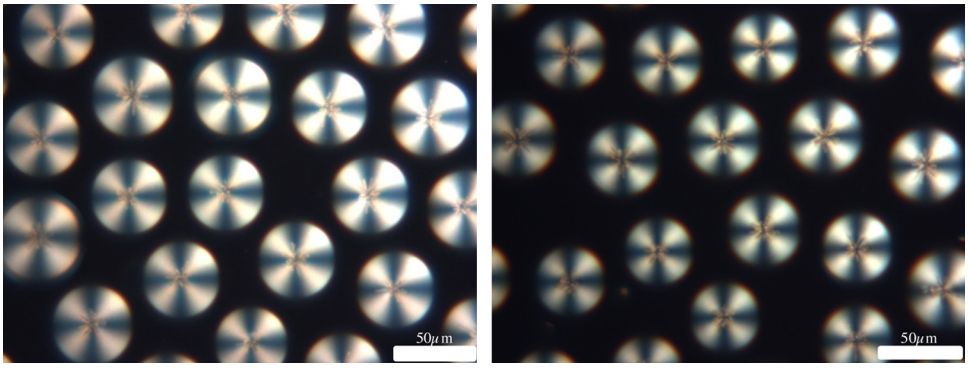


Fig. 2. POM images of $5CB_{PAA}$ (left) and $5CB_{PAA\text{-}biotin}$ (right) in water.

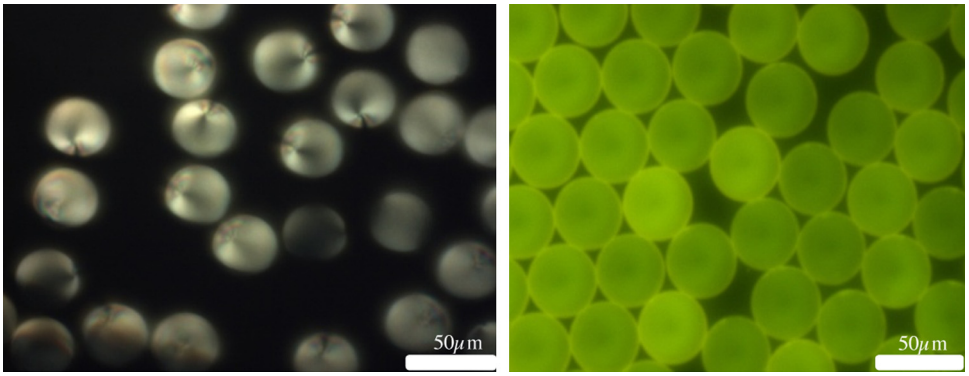


Fig. 3. $5CB_{PAA\text{-}biotin}$ droplets in a 100 μg/mL avidin solution under cross polarizers (left) and in a 100 μg/mL $avidin_{rhodamine\ 6G}$ solution under fluorescence (right).

2.5. Measurements

The on-chip formation of droplets was imaged using an STC-TC83USB-AS camera (Sen Tech, Japan) attached to the inverted microscope. The images of the droplets were captured using a POM (Leitz, ANA-006, Germany) under crossed polarizers using a CCD camera (Samwon, STC-TC83USB, Korea). The $avidin_{rhodamine\ 6G}$ on the $5CB_{PAA\text{-}biotin}$ droplets was confirmed by fluorescence microscopy (Nikon Eclipse, E600POL, Japan).

3. Results and discussion

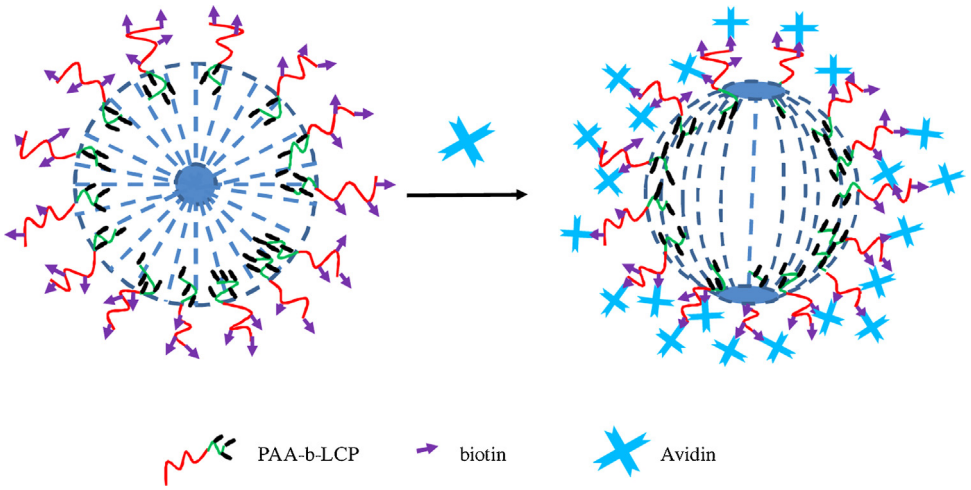
3.1. Biotinylation of $5CB_{PAA}$ droplets and their responses to avidin

Fig. 2 shows a POM image of the $5CB_{PAA}$ droplets under cross polarizers. The droplets exhibited radial orientation corresponding to homeotropic anchoring of 5CB. The 5CB droplets without any coating on the surface are known to exhibit a bipolar configuration in the aqueous medium [13]. The radial orientation of the $5CB_{PAA}$ droplet is due to the negative charge produced by the deprotonated carboxylic groups of the PAA (pK_a 4.7) chains at pH 7 [13]. Fig. 2 (right) shows a POM image of the $5CB_{PAA\text{-}biotin}$ droplets. The radial configuration of the droplets is preserved after biotinylation. After biotinylation of the PAA chains, the biotin moiety also exhibits negative charges because the pK_a of biotin is 4.8. Thus, the negative charge states of PAA and biotin contribute to the radial orientation of the $5CB_{PAA\text{-}biotin}$ droplets because of an electric field generated by these charges at the 5CB/aqueous interface. The radial orientation of $5CB_{PAA\text{-}biotin}$ changed to a bipolar orientation when the aqueous medium was replaced with a 100 μg/mL avidin solution, as shown in Fig. 3. In the radial configuration the 5CB molecules are oriented perpendicular to the droplets surface with a defect at the core of the droplets whereas, in the bipolar configuration, the 5CB molecules are oriented parallel to the surface of the droplets with two diametrically opposite surface point defects at the poles of the droplets. This R-B change may be due to the formation of a complex between the biotin bonded to the PAA chains and the avidin at the 5CB/aqueous interface as shown in Scheme 1. A similar LC orientation change was observed when the PE on the 5CB formed complexes with oppositely charged proteins [13,25]. After the formation of the complex with protein, the electric field generated by the charged PE is reduced on the LC side because of the neutrality of the complex. This reduced electric field causes the R-B orientation change of 5CB at the 5CB/aqueous interface.

Biotin–avidin complex formation on the 5CB/aqueous interface was confirmed with a 100 μg/mL $avidin_{rhodamine\ 6G}$ solution. Fig. 3 shows the fluorescence image of the $5CB_{PAA\text{-}biotin}$ droplets after complexation with $avidin_{rhodamine\ 6G}$. The image shows green droplets, suggesting that $avidin_{rhodamine\ 6G}$ was adsorbed successfully on the $5CB_{PAA\text{-}biotin}$ droplets. As a control, this receptor/ligand system was tested with the $5CB_{PAA}$ droplets without biotinylation. A 100 μg/mL avidin solution was reacted with the $5CB_{PAA}$ droplets under the same experimental conditions. The initial radial configuration was maintained as shown in Fig. 4, indicating that avidin did not bind to the $5CB_{PAA}$ droplets due to absence of the receptor. Thus, the R-B change of the $5CB_{PAA\text{-}biotin}$ droplets in the avidin solution was due to the formation of the complex between biotin and avidin at the 5CB/aqueous interface.

3.2. Effects of biotin concentration on avidin detection

To determine the optimum condition for biotinylation, the R-B change of the $5CB_{PAA\text{-}biotin}$ droplets biotinylated with different concentrations (C_bs) were tested with a 100 μg/mL avidin solution. Fig. 5 shows the POM images of the $5CB_{PAA\text{-}biotin}$ droplets biotinylated at $C_b = 5$ μg/mL after injecting a 100 μg/mL avidin solution.



Scheme 1. Radial to bipolar transition of the $5CB_{PAA\text{-}biotin}$ in an avidin aqueous solution.

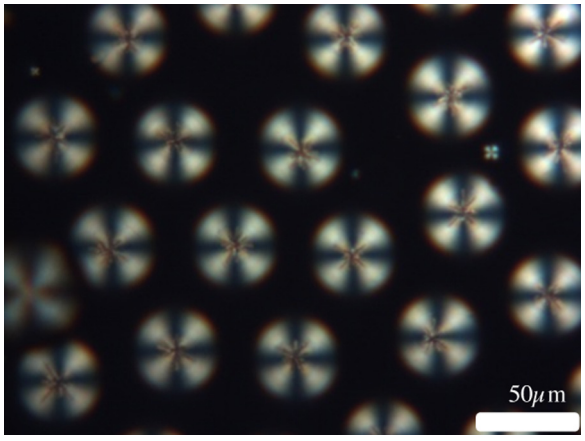


Fig. 4. POM image of $5CB_{PAA}$ droplets in a 100 μg/mL avidin solution.

The initial radial orientation of the $5CB_{PAA\text{-}biotin}$ droplets was maintained, indicating that not enough biotinylation occurred for the R-B change. When C_b was increased to 7.5 μg/mL, most of the droplets changed from the radial to preradial orientation. When C_bs were 10, 50, 75, and 100 μg/mL, a complete R-B configurational change of the 5CB droplets was observed. Since a clear visible R-B change was observed at C_b = 100 μg/mL, all the experiments were performed with the $5CB_{PAA\text{-}biotin}$ droplets biotinylated with a 100 μg/mL biotin solution.

3.3. Sensitivity of $5CB_{PAA\text{-}biotin}$

Fig. 6 shows the POM images of the $5CB_{PAA\text{-}biotin}$ droplets in the avidin solutions at different concentrations (C_As). The initial radial orientation did not change at C_A = 0.1 μg/mL. At C_A = 0.5 and 0.75 μg/mL, the orientation of a few droplets appeared to change from the radial to preradial configuration. The change to preradial configuration become more visible at C_A = 0.8 and 0.9 μg/mL. At C_A = 1 and 10 μg/mL, the droplets exhibit an axial configuration with a defect ring located on the surface of a drop along one of

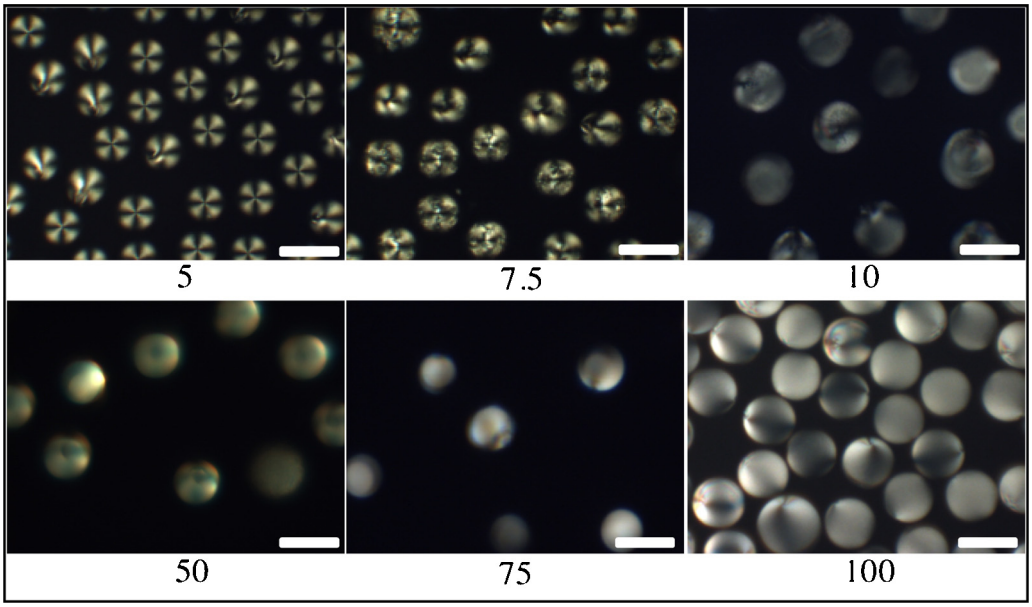


Fig. 5. POM images of $5CB_{PAA\text{-}biotin}$ droplets biotinylated at different C_bs in a 100 μg/mL avidin solution. The digit under each image represents C_b in μg/mL. All scale bars are 50 μm.

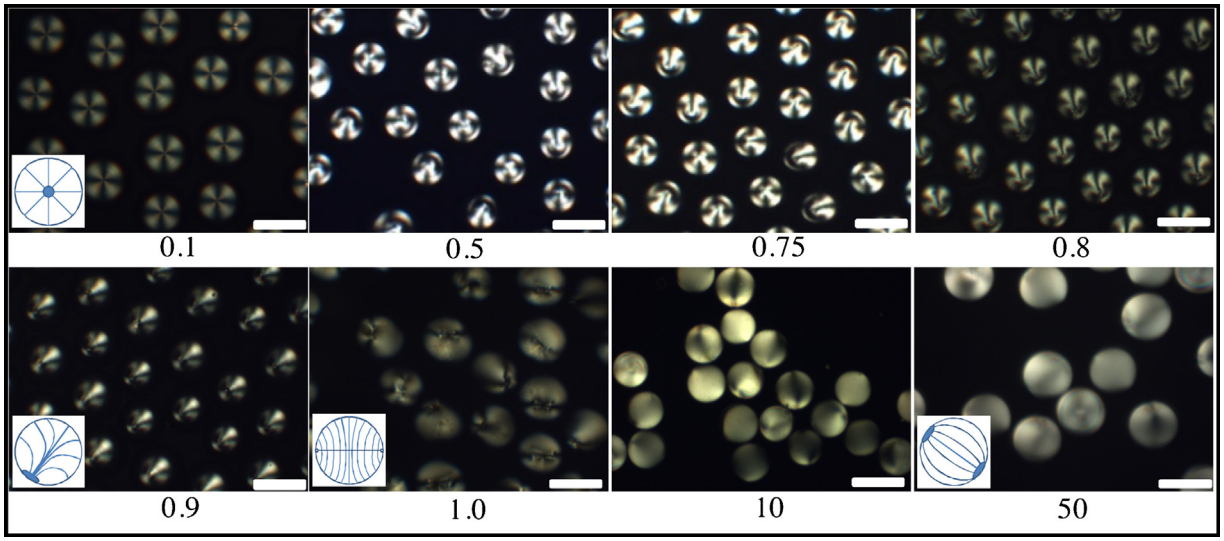


Fig. 6. POM images of $5CB_{PAA\text{-}biotin}$ droplets in different concentration (C_A) of avidin solutions. The digit under each image represents C_A in μg/mL. Insets represent the direct field in the droplet. All scale bars are 50 μm.

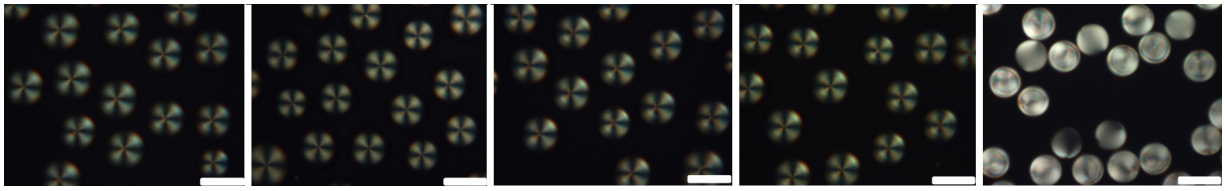


Fig. 7. POM images of $5CB_{PAA\text{-}biotin}$ droplets in 100 μg/mL aqueous solution of BSA, Lyz, Hb, ChTg, and a mixture of ChTg and avidin (left to right). All scale bars are 50 μm.

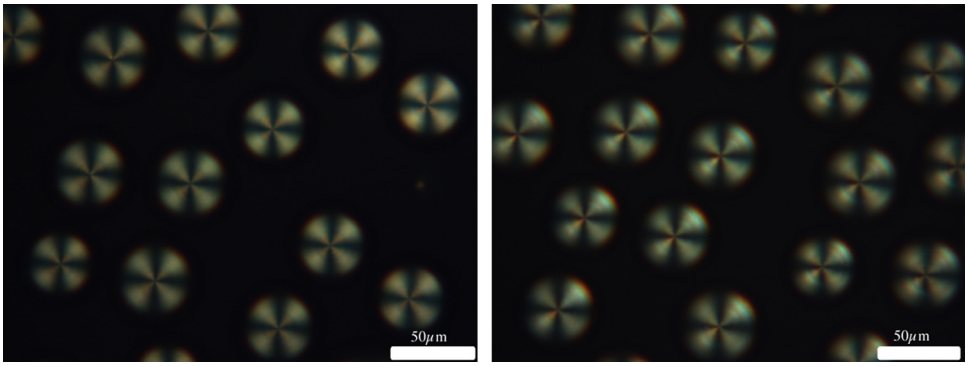


Fig. 8. POM images of $5CB_{PAA\text{-}biotin}$ droplets after five days (left) and after one month (right).

its great circles [4]. At $C_A \geq 50$ μg/mL, a clear bipolar configuration was observed. Thus, the sequence of radial, preradial, axial, and bipolar was observed as C_A increased. This sequence is usually observed when the anchoring of the LC droplet changes from tangential to homeotropic [26]. For example, the bipolar droplets transform into a transient axial droplet characterized by an equatorial disclination line. Subsequently, this line gradually shrinks and eventually disappears, leading to the formation of a point defect (preradial), which then migrates to the droplet center to form the final radial droplet. Thus, the observation indicates that, with increased C_A, the biotin–avidin binding changes the anchoring condition, and the amount of binding reflects the type of configuration observed in the LC droplet. These in-depth studies on the droplet configurations find that the $5CB_{PAA\text{-}biotin}$ biosensor can effectively detect the avidin with concentrations (C_As) ≥ 0.5 to 10 μg/mL. Thus, studies on the change of the director configurations of the $5CB_{PAA\text{-}biotin}$ droplets by the avidin–biotin binding may provide a new platform for the quantitative analysis of the analytes.

The $5CB_{PAA\text{-}biotin}$ droplets have enhanced sensitivity (0.5 μg/mL avidin) compared to other reported values for protein detection at the 5CB/aqueous interface. For example, the poly(dimethylaminoethylmethacrylate) (PDMAEMA)-coated 5CB in a TEM grid could measure a 10 μg/mL BSA aqueous solution [25]. The quarternized poly(4-vinylpyridin) (QP4VP) brushes at the 5CB/aqueous interface enabled the detection of ∼100 μg/mL protein solutions [27], and the PAA brushes detected a 32 μg/mL lysozyme solution [2]. The low sensitivity observed in this study may be due to the strong affinity of avidin to biotin.

3.4. *Specificity and stability of $5CB_{PAA\text{-}biotin}$*

The $5CB_{PAA\text{-}biotin}$ droplets were tested for their specificity to avidin. The POM images of the $5CB_{PAA\text{-}biotin}$ droplets in a 100 μg/mL

avidin solution under crossed polarizers exhibited bipolar orientation upon binding between biotin and avidin, as discussed in the previous section (Fig. 3). Other proteins were tested to check whether their binding with biotin is possible. Fig. 7 shows that the initial radial orientation of the $5CB_{PAA\text{-}biotin}$ droplet was maintained in 100 μg/mL BSA, Lyz, Hb, and ChTg solutions, indicating that other proteins did not bind to the biotin on the LC droplet. A protein mixture of avidin and ChTg was also tested. An R-B change was observed after introducing the 100 μg/mL mixture solution of avidin and ChTg. Thus, this result suggests that selective binding is possible with the introduction of a receptor on the LC droplet. Fig. 8 shows the POM images of $5CB_{PAA\text{-}biotin}$ droplets after five and thirty days. The initial radial orientation and shape of the droplet preserved which may be due to the presence of stable charges on the droplet surface; indicate the high stability of these droplets. To the best of our knowledge, this result is the first demonstration of a biosensor using LC droplets produced with microfluidics. This platform can potentially be expanded to other ligand/receptor systems for other biomedical applications.

4. Conclusion

Herein, $5CB_{PAA}$ droplets were biotinylated using EDC·HCl and NHS coupling agents at the 5CB/aqueous interface. This $5CB_{PAA\text{-}biotin}$ biosensor could detect small amounts of avidin (0.5 μg/mL) through the R-B configuration change using POM under crossed polarizers. The low detection limit, stability, specific detection, sequential configurational change, high activity, and high sensitivity of the $5CB_{PAA\text{-}biotin}$ droplet suggest that these receptor-coupled LC droplets may effectively be used as a new platform for developing a biosensor without the aid of sophisticated instruments for detection.

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