# Label-free protein quantitation using liquid crystal-enhanced optofluidic biosensor

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## ABSTRACT

Protein detection plays an important role in the medical research. Liquid crystals (LCs), as a class of sensitive materials, exhibit a promising ability in the biosensing field. Herein, we exploited an ultrasensitive biosensor for protein detection, employing the whispering-gallery-mode (WGM) from the LC-amplified optofluidic micro-resonator. The biomolecules can trigger both light-matter interactions and the orientation transitions of LC molecules. The WGM spectral wavelength shift was recorded as the sensing indicator, and a detection limit of 15 fM for bovine serum albumin (BSA) was achieved. Our LC-amplified optofluidic biosensor provides a new solution for the ultrasensitive, real-time, and stable biological detection.

**Keywords:** Liquid crystal, Biosensor, Protein assay, Whispering-gallery mode, Optofluidic

## 1. INTRODUCTION

Proteins play critical roles in the regulation of metabolism. Based on their great importance in biological activities and disease diagnoses in indicating pathological biological conditions, it is essential to quantify proteins with high sensitivity and selectivity.1-3 Especially during current pandemic period, IgG and its anti-body can serve as reliable biomarkers to prevent and control the virus infection at an early stage.4 Current approaches illustrate various capabilities in detecting protein and remain limitations due to high-cost and complex operations. A low-cost, ultra-sensitive and stable sensing system for protein assay is urgent and necessary.

Liquid crystals (LCs) acting as a promising branch of quickly-responsive, highly-sensitive, and low-cost materials, have been exploited in a wide variety of biosensing applications.5-8 Relied on orientation transitions of LC molecules, the most popular detection method of LC-based biosensors is the polarized optical microscope (POM) based observation.9, 10 To replace and overcome the difficulties of the naked eye observation, some researchers employed the image processing technique, UV-vis spectra, and reflection spectra to enhance the sensitivity of LC-based biosensors.11-13 With the merits of high signal intensity and a narrow laser linewidth from whispering gallery mode (WGM),14-16 an effective and highly sensitive detection arises from the combination of LC droplets and WGM laser emission spectra. However, the variation in the external environment (e.g., the evaporation of the LC immersed liquid medium) can seriously affect the sensitivity and the stability of such sensing platforms.17

In this work, taking advantages of high Q factor, small mode volume, and strong evanescent field from passive WGM cavities, we developed a LC-amplified resonator for ultrasensitive biological detection. The microcavity (with a shape of microbubble) provides a WGM ring resonator for LCs. Biomolecules attached to the internal surface of the microcavity can trigger the orientation transition of LC molecules. Owing to a thin wall of the microcavity, both the light-matter interactions and the alignment variation of LC molecules can be captured by the WGM spectrum to enhance

the biomolecular signal. With the triple effects of the biomolecular polarization, the orientation transition of LC molecules, and the WGM, small protein variations can be monitored and result in the wavelength shift. A detection of femtomole-level BSA concentration can be achieved. Compared to the conventional POM, the sensitivity has been improved by six orders of magnitude, via the use of LC-amplified WGM biosensors. Besides the sensing capability in protein assays, specific biosensing for IgG and Anti IgG was also studied. Overall, our platform exhibits a multi-functional approach for label-free, ultra-sensitive, and real-time protein detection. Furthermore, according to the wide applications of LCs in the biosensing field, such a promising platform can also be extended for detecting other types of biomolecules by changing the decoration of the microfluid internal surface.

## 2. RESULTS AND DISCUSSIONS

The effect of protein molecules on the LC-amplified optofluidic biosensor was exploited. The DMOAP, as an alignment layer, can promote the homeotropic orientation of LC molecules. After the addition of biomolecules, the anchoring force from DMOAP will be suppressed by protein molecules, which results in the alignment transition of LC molecules from homeotropic to planar, as shown in Figure 1. The orientation transition of LC molecules could lead to the change of the WGM spectra.

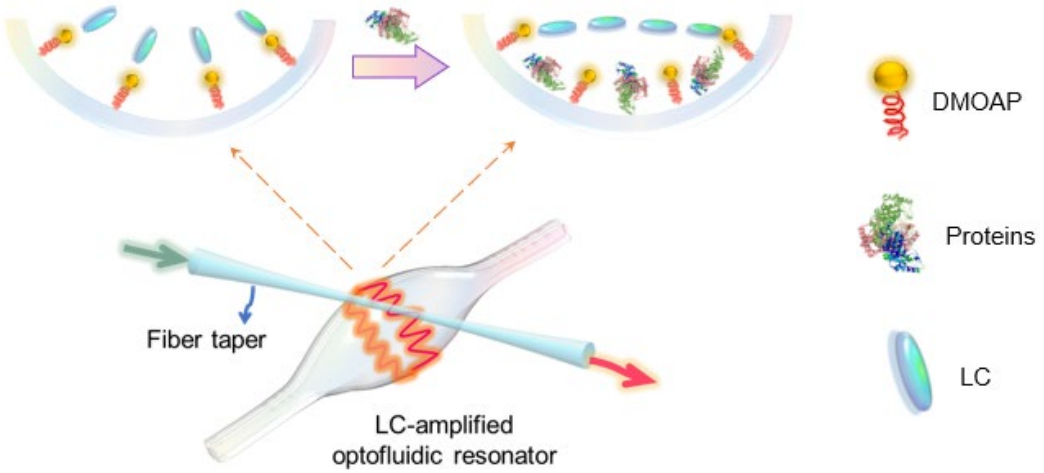


Figure 1 Under the disturbance of BSA molecules, the ordering of LC in the micro-resonator is changed from homeotropic to planar.

BSA was taken as the test sample and the variation of WGM spectra were monitored. Under various concentrations of BSA, spectral responses all showed red-shift at first and then displayed blue-shift tendency, as seen in Figure 2a. This is because the resonance frequency is sensitive to the change in the close vicinity of the cavity interface, e.g. biomolecular random absorption behavior and the re-orientation of LC molecules. On one hand, the resonant wavelength of WGMs scales proportionally with the BSA surface density. The light-matter interactions between the evanescent field and biomolecules will lead to the excess polarizability, the energy perturbation of a single-photon resonant state, and the red-shift tendency. On the other hand, the WGM of this LC-amplified optofluidic biosensor conformed to the transverse magnetic (TM) mode. For the initial state, the oscillating electric field along the long axis of LC molecules with the homeotropic orientation followed the extraordinary refractive index ($n_e$). With the disturbance of biomolecules, the TM mode will respond to the refractive index of LCs from the extraordinary refractive index to the ordinary refractive index, which triggered a blue-shift tendency on the spectrum. Accordingly, total wavelength shifts of WGM spectra (absolute value of red shifts + absolute value of blue shifts) were employed to characterize BSA concentrations. Signal responses under various concentrations of BSA (from $10^{-12}$ g/ml to $10^{-3}$ g/ml) were monitored respectively, each for 15 mins, as seen in Figure 2b. There existed a linear relationship between the total spectral wavelength shifts and the BSA concentrations. This indicates that the minor variation of BSA can now be easily captured via LC orientation transitions and WGM spectra.

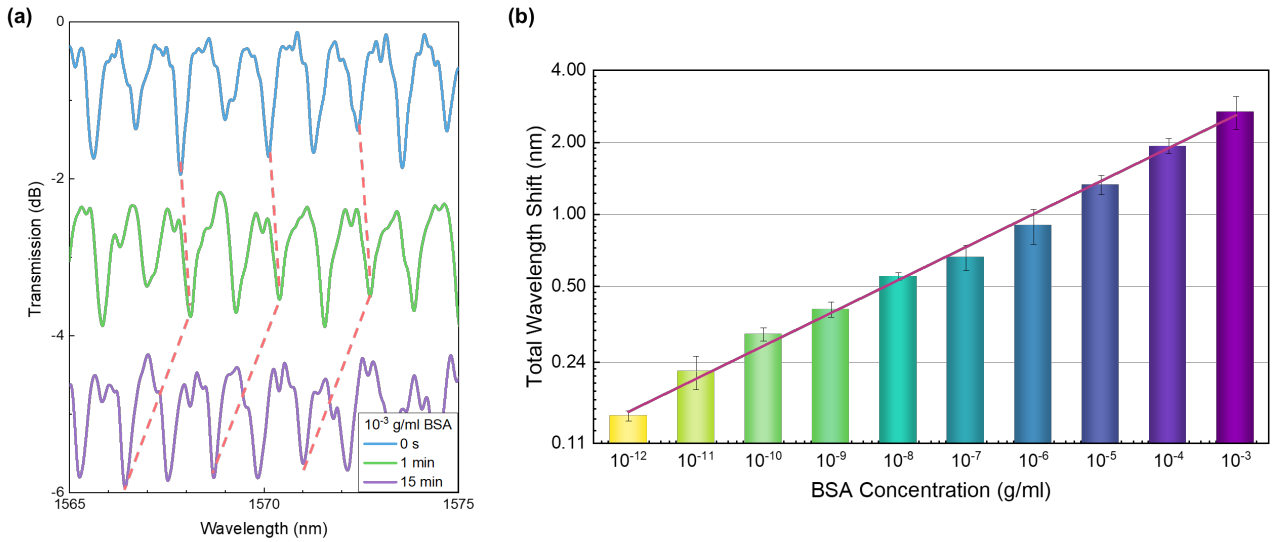


Figure 2 WGM spectra of the LC-amplified optofluidic sensor under (a) $10^{-3}$ g/ml BSA (at 0, 1, 15 mins, respectively). (b) The overall wavelength shift responses from WGM spectra under different BSA concentrations (from $10^{-12}$ to $10^{-3}$ g/ml).

To verify the generic applicability of the LC-amplified optofluidic biosensor on protein detection, human IgG (HIgG) was also tested in this work. As seen in Figure 3, the total wavelength shifts of spectral responses depend linearly on the HIgG concentrations, which showed a similar trend as those in the BSA sensing. According to the above results, our biosensing system exhibited a great potential in the ultra-sensitive detection of various protein molecules.

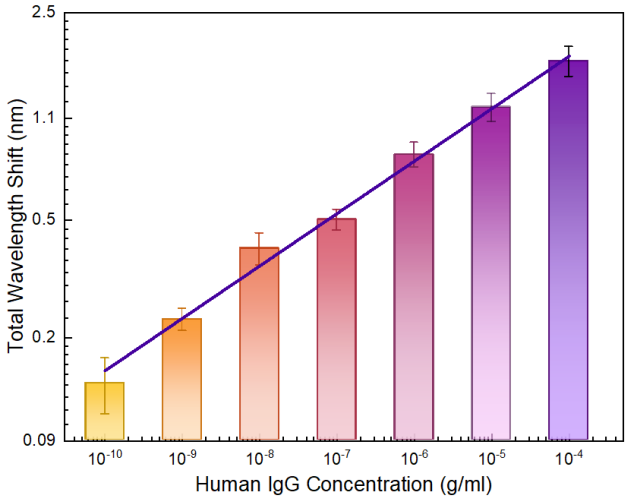


Figure 3 The overall wavelength shift responses under various concentrations of human IgG.

Moving a step forward, we have also investigated the high sensitivity and specific biosensing ability of our sensing platform by employing the antigen-antibody binding events between HIgG and anti-human IgG (AHIgG). 1 mg/ml HIgG was used as a substrate to modify the inner surface of the microcavity. BSA was used to check the unbound sites. Subsequently, HIgG-modified microcavity was exposed to PBS solution (as a blank group), anti-human albumin (anti-HSA), anti-mouse IgG and AHIgG with the same concentration of $10^{-3}$ g/ml, respectively. Compared to the control group (HIgG + PBS solution), only the AHIgG group exhibited an obvious wavelength response, as seen in Figure 4a. We also tested and recorded different concentrations of AHIgG via spectral wavelength shifts, in Figure 4b. All responses for detecting AHIgG should exceed 2.13 nm, because HIgG (as the substrate) was decorated on the interface. This indicates that LC-amplified microfluidic shows high selectivity, which can be used for the specific and highly sensitive detection of various proteins, especially for biomarkers.

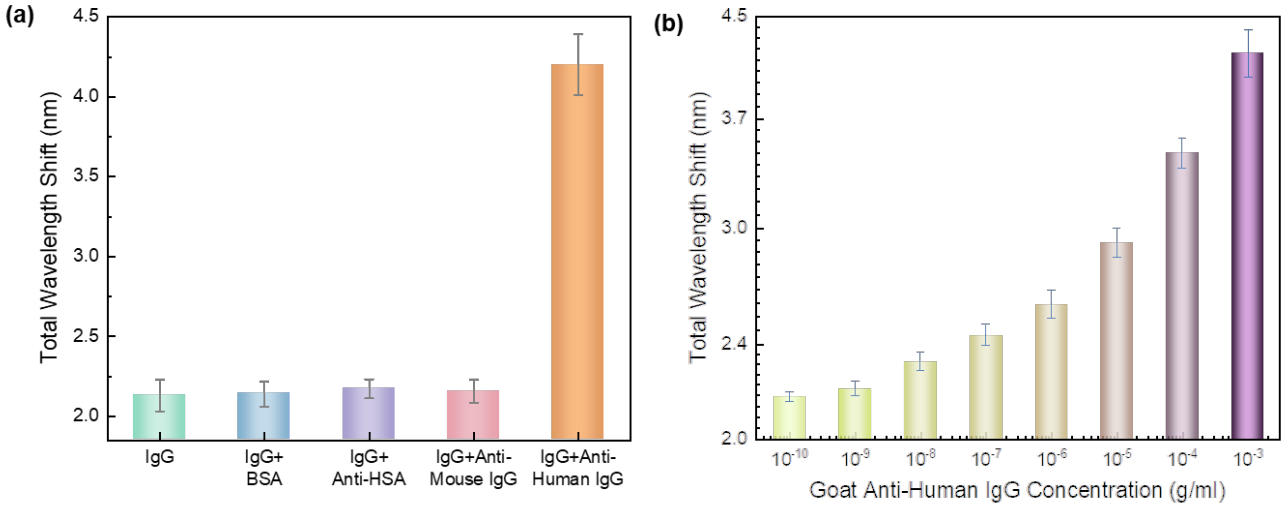


Figure 4 Effects of specific binding events between HIgG and AHIgG. (a) wavelength shift responses when LCs were exposed to $10^{-3}$ g/ml proteins (BSA, anti-HSA, anti-mouse IgG, and anti-human IgG). (b) WGM spectral shift resulted from antibody-antigen binding events between $10^{-3}$ g/ml Human IgG and different concentrations of anti-human IgG.

## 3. CONCLUSION

In this work, an ultra-sensitive LC-amplified optofluidic resonator biosensor has been developed, based on WGM spectra, for the analysis of proteins. The amplified optofluidic resonator was employed to provide a stable and reliable ring resonator for LCs and to support the WGM. The protein decorated on the internal surface of the DMOAP-coated micro-resonator triggered the orientation transition of LC molecules. Due to the interaction between biomolecules and the laser beam, the RI of protein molecules was changed accordingly. Relying on joint effects from these two factors, WGM spectra can amplify and characterize the biomolecular concentration via the wavelength shift. When BSA was taken as a test sample, the total spectral shift scaled linearly with the BSA concentrations. Other protein molecules (human IgG) and specific antibody-antigen binding experiments were also exploited to demonstrate the applicability and selectivity of our sensing platform for protein detection. Due to the wide applications of LCs in the biosensing field, in addition to protein assays, this LC sensing system can also provide promising solutions to the label-free detection of other biomolecules by simply changing the decoration of microfluidic resonators.

## 4. MATERIALS AND METHODS

### 4.1 Fabrication of micro-resonator and fiber taper

For ultra-sensitive sensing, we developed a LC-amplified WGM resonator. The micro-resonator was produced from a silica-based microbubble and LCs (as amplifiers) filled in the cavity. The preparation of the microbubble can be separated into two steps. Firstly, the silica capillary is stretched under the oxyhydrogen flame with a diameter at centimeter scale. Then, the microbubble is formed by the expanded capillary with the assistance of a smaller hydrogen oxide flame (diameter at millimeter level) and constant pressure. By changing the stretching length and flame, the outer diameter and wall thickness can be adjusted. In this research, the microbubble with a diameter of 210 μm and a wall thickness of ~ 3 μm was fabricated. Based on the heating-stretching technique, a fiber taper with a diameter of 1 ~ 2 μm was formed to excite the WGM.

### 4.2 Functionalization of DMOAP and protein molecules

0.01% (v/v) of DMOAP solution was filled into the microcavity for 30 mins to form the homeotropic alignment layer at the internal surface of the microcavity. Deionized water was used to remove the unbound DMOAP molecules. Then, for the immobilization of biomolecules, protein solutions were injected into the DMOAP-decorated micro-resonator for 30

mins, and excessive biomolecules were removed. Finally, LCs were filled into the micro-resonator at a flow rate of 0.7 μL/min for the protein sensing.

### 4.3 Optical Setup

A tunable laser was used as the pump source and was coupled to the micro-resonator via the fiber taper. Relying on the high refractive index of LC molecules ($n_o$=1.51, $n_e$=1.67) and the thin wall of the micro-resonator, the evanescent field can be coupled into the optofluidic resonator and can be employed to monitor the changes of LCs.

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