



Label-free liquid crystal-based aptasensor for sensitive detection of interleukin-6 via UV-induced 4-cyano-4'-biphenylcarboxylic acid functionalization

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ABSTRACT

Interleukin-6 (IL-6) is a multifunctional cytokine involved in immune regulation and inflammatory diseases. Thus, its rapid and sensitive quantification is clinically important. However, conventional immunoassays often require labels, complex instrumentation, and lengthy workflows. In this study, we present a label-free liquid-crystal (LC) aptasensor that converts IL-6 binding into a robust optical response. The nematic 4-cyano-4'-pentylbiphenyl was partially UV-oxidized to 4-cyano-4'-biphenylcarboxylic acid. This oxidation introduced carboxyl groups to facilitate EDC/NHS coupling to an amino-terminated complementary oligonucleotide embedded in the LC phase. The tail region of an IL-6-specific aptamer hybridized with the surface-anchored complementary oligonucleotide (complementary strand) and disrupted homeotropic anchoring in the absence of IL-6, yielding a bright planar texture; IL-6 binding prevents hybridization and preserves a dark homeotropic texture. The percent brightness from polarized optical microscopy correlated linearly with log[IL-6] between 10 pg·mL⁻¹ and 10 ng·mL⁻¹, with a detection limit of 4.8 pg·mL⁻¹. The aptasensor showed high specificity against common interferents and recoveries ranging from 97.22 to 100.65% in spiked human serum. This simple, rapid (≤30 min), and low-cost platform is readily adaptable for multiplex cytokine analysis.

1. Introduction

Interleukin-6 (IL-6) is a pleiotropic cytokine that plays essential roles in immune regulation, inflammation, and tumor progression. Elevated IL-6 levels strongly correlate with severe inflammatory and infectious diseases, including sepsis, rheumatoid arthritis, and coronavirus disease 2019 (COVID-19). Thus, IL-6 is a crucial biomarker for early disease diagnosis and monitoring [1,2]. Under physiological conditions, serum IL-6 concentrations are typically below 15 pg·mL⁻¹ but can increase to several ng·mL⁻¹ during acute inflammatory responses [3,4]. Therefore, sensitive and rapid IL-6 quantification in biological samples is vital for the clinical assessment of immune dysfunction [5,6].

Current strategies for interleukin-6 (IL-6) detection can be systematically categorized according to two independent criteria: (i) the molecular recognition element responsible for target binding and (ii) the signal transduction mechanism used to convert the recognition event into a measurable output. Antibody-based assays remain the most

established approaches for IL-6 quantification, including enzyme-linked immunosorbent assays (ELISA) [7,8], chemiluminescent immunoassays [9,10], and radioimmunoassays [11], which provide high sensitivity but typically require labeled reagents, multistep washing procedures, and centralized instrumentation; label-free antibody-based platforms such as surface plasmon resonance (SPR) and surface-enhanced Raman scattering (SERS) immunosensors have also been developed to simplify assay formats while maintaining strong binding affinity [12,13]. In parallel, aptamer-based biosensors have emerged as attractive alternatives to antibody-based systems due to their chemical stability, synthetic accessibility, and facile modification, and IL-6 aptasensors have been reported using diverse transduction schemes including electrochemical [14], fluorescence, SPR, and SERS-based platforms [15,16]; nevertheless, many of these approaches still suffer from fabrication complexity, susceptibility to matrix interference, or reliance on sophisticated instrumentation. Beyond optical methods, purely electrical label-free strategies, such as field-effect transistor (FET)-based sensors and non-

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faradaic impedance spectroscopy [17–19], have demonstrated rapid response and ultra-low detection limits for IL-6 monitoring, although their practical deployment is often constrained by complex device fabrication and stringent electrical noise control. Distinct from the above approaches, liquid crystal (LC)-based biosensors exploit the intrinsic sensitivity of LC orientation to subtle interfacial energy changes, enabling optical amplification of molecular recognition events without labels or complex surface architectures; however, LC-based aptasensing strategies for IL-6 detection remain scarce, motivating the development of the present LC-based aptasensor.

Liquid crystals (LCs) have emerged as versatile transduction materials that can convert interfacial molecular interactions into optical signals. Their anisotropic alignment and high sensitivity to interfacial perturbations enable label-free real-time detection of biomolecular events [20,21]. In LC-based biosensors, specific adsorption or recognition at the aqueous/LC interface alters molecular orientation, consequently producing a detectable transition between homeotropic (dark) and planar (bright) textures under polarized optical microscopy (POM) [20–22]. However, nonselective adsorption and the lack of bio-recognitive elements limit the molecular specificity of conventional LC sensors; this restricts their application in complex biological matrices [23–26].

To address this challenge, aptamers—synthetic oligonucleotides with high affinities and specificities—have been integrated into LC systems for selective molecular recognition [27,28]. Aptamer–LC hybrid sensors have been reported for proteins, ions, and small molecules. However, their application in cytokine detection remains scarce. Previous LC-based platforms for IL-6 analysis typically relied on surface-deposited nanostructures or antibody-functionalized interfaces, which often involved multistep surface modifications leading to poor optical stability [29–31].

Herein, a label-free LC-based aptasensor is introduced for the ultra-sensitive detection of IL-6 through ultraviolet (UV)-induced carboxylation of the nematic LC 4-cyano-4'-pentylbiphenyl (5CB). The in-situ oxidation of 5CB generates 4-cyano-4'-biphenylcarboxylic acid (CBA) with carboxyl groups that are covalently linked to an amino-modified complementary oligonucleotide (5'-AmMC6/TTT CCC CCA AAA A-3') through EDC/NHS coupling. The resulting complementary oligonucleotide-functionalized LC phase enables hybridization-controlled optical switching regulated by IL-6–aptamer interactions (5'-CTT CCA ACG CTC GTA TTG TCA GTC TTT AGT TTT TTT GGG GGA AAA A-3') [5]. In this system, the aptamer functions as the molecular recognition element for IL-6, whereas the surface-anchored complementary oligonucleotide serves only to reversibly hybridize with the aptamer and modulate interfacial free energy. Previous studies combining molecular dynamics simulations and surface plasmon resonance imaging have demonstrated that this IL-6 DNA aptamer exhibits strong and stable binding to IL-6, characterized by persistent intermolecular hydrogen bonding, compact complex formation, and sustained SPR responses, thereby confirming its high binding affinity toward IL-6 [32]. This strategy eliminates surface pre-coating steps, enhances chemical stability, and provides a robust optical transduction mechanism. The developed system demonstrates high sensitivity, specificity, and reliable detection in spiked human serum, highlighting its potential as a rapid and low-cost platform for cytokine quantification and point-of-care diagnostics.

2. Materials and methods

2.1. Materials and reagents

Nematic liquid crystal 5CB was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Glass microscope slides (76 × 26 mm, 1.0 mm thickness) were obtained from Matsunami Glass Ind., Ltd. (Osaka, Japan). Copper transmission electron microscopy (TEM) grids (75 mesh, pitch = 340 μm, hole = 285 μm, bar = 55 μm) were purchased

from Gilder Grids (Grantham, UK). Sulfuric acid (H₂SO₄, ≥95%), hydrogen peroxide (H₂O₂, 30%), methylene chloride (CH₂Cl₂, ≥99.5%), octyltrichlorosilane (OTS, ≥97%), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were obtained from Daejung Chemicals & Metals Co., Ltd. (Siheung, Korea). Ultrapure deionized (DI) water with a resistivity of 18.2 MΩ·cm was produced using a Milli-Q purification system (Millipore, Billerica, MA, USA).

A human IL-6 ELISA Kit, human serum, EDC, NHS, and IL-6 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Additional proteins and enzymes used in selectivity experiments, including L-lysine (L-Lys), interleukin-5 (IL-5), interleukin-4 (IL-4), interferon-gamma (IFN-γ), hemoglobin (Hgb), carcinoembryonic antigen (CEA), mucin 1 (MUC1), mucin 4 (MUC4), mucin 16 (MUC16), and immunoglobulin B (IgB), were also obtained from Sigma-Aldrich.

The amino-modified complementary oligonucleotide sequence (5'-NH₂-C6-TTT TTT CCC CCA AAA A-3') and the IL-6-specific aptamer (5'-CTT CCA ACG CTC GTA TTG TCA GTC TTT AGT TTT TTT GGG GGA AAA A-3') were synthesized and supplied by Integrated DNA Technologies Pte Ltd. The phosphate-buffered saline (PBS) solution (10 mM phosphate, 138 mM NaCl, 2.7 mM KCl; pH 7.4) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). All the reagents were used as received without further purification.

2.2. UV oxidation of 5CB to CBA

Nematic liquid crystal 5CB was photo-oxidized under UV irradiation to form CBA, thereby introducing reactive carboxyl (–COOH) groups for subsequent covalent coupling [33]. Briefly, 200 mg of 5CB was placed in a quartz Petri dish and spread into a thin uniform layer. The dish was exposed to UV light (254 nm, 8 W) at a distance of 5 cm for 60 min in an open-air environment to facilitate photochemical oxidation. The terminal pentyl chain of 5CB was oxidized during this process, yielding the carboxylated derivative CBA.

The UV irradiation conditions (wavelength, intensity, exposure time, and distance) were strictly controlled to ensure consistent interfacial oxidation. Although the UV treatment produces a partially oxidized LC mixture rather than a single pure compound, the resulting carboxylated LC phase exhibits reproducible interfacial properties and optical responses under identical preparation conditions.

2.3. Fabrication of CBA-functionalized LC complementary strand

To prepare the complementary oligonucleotide-functionalized LC phase, the synthesized CBA was activated using EDC/NHS coupling chemistry to form covalent linkages with amino-terminated oligonucleotides. Briefly, 10 mg of CBA was dissolved in 1 mL of ethanol/water (1:1 v/v), followed by the addition of 10 mM EDC and 25 mM NHS. The mixture was gently stirred at room temperature for 30 min to form an active NHS–ester intermediate. Subsequently, 10 μL of the amino-modified complementary oligonucleotide sequence (5'-AmMC6/TTT TTT GGG GGAA AAA-3') was added to the activated CBA solution and incubated for 2 h at 25 °C to allow amide bond formation between the carboxyl group of CBA and the amine terminus of the oligonucleotide.

After conjugation, the reaction mixture was centrifuged to remove unreacted reagents, and the resulting CBA–complementary strand conjugate was washed thrice with PBS. The purified complementary strand complex was re-dispersed in 5CB to yield a homogeneous mixture at a concentration of 0.5 wt% complementary strand–CBA in the LC matrix. The mixture was sonicated for 5 min to ensure a uniform distribution and then degassed under vacuum for 1 h to remove trapped air bubbles.

2.4. Assembly of LC optical cell

Glass slides were cleaned (piranha, 7:3 v/v H₂SO₄/H₂O₂, 80 °C, 1 h), rinsed, dried under N₂, and dehydrated at 120 °C overnight. Slides were

silanized in 0.2% (v/v) OTS in anhydrous n-heptane (25 °C, 30 min), rinsed (CH₂Cl₂, ethanol), N₂-dried, and cured at 120 °C for 1 h to yield a stable hydrophobic monolayer. The OTS-coated substrates were used to support copper TEM grids and provide homeotropic anchoring for the 5CB-based films.

2.5. Detection procedure

IL-6 was detected using the prepared complementary strand-functionalized LC films supported on the copper TEM grids. The procedure consisted of three main stages: (1) pre-incubation of the IL-6 aptamer with the analyte, (2) application of the sample to the aqueous/LC interface, and (3) optical observation and quantification of the LC response.

First, the IL-6-specific aptamer was diluted to the desired concentration in PBS. A defined volume of IL-6 solution at various concentrations (ranging from 10 pg·mL⁻¹ to 10 ng·mL⁻¹) was added to the aptamer solution and gently mixed. The mixture was incubated at room temperature for 30 min to allow for specific binding between IL-6 and the recognition domain of the aptamer.

Next, 1 µL of the complementary strand-functionalized LC mixture was introduced into each well of a chambered OTS-coated glass slide containing a copper TEM grid. Excess LC was removed using a microcapillary tube to obtain uniform films within each grid opening. The LC-filled grids were then immersed in 200 µL of the pre-incubated aptamer-analyte solution, ensuring complete contact between the aqueous phase and LC interface. The system was left undisturbed for 15 min to allow hybridization or binding events to influence interfacial LC alignment.

2.6. Selectivity and real sample testing

Selectivity was evaluated against physiologically relevant interferents (e.g., L-lysine, IL-4/IL-5, IFN-γ, Hgb, CEA, MUC1/4/16, and IgB; each 10 ng·mL⁻¹ in PBS). For each test, the aptamer was pre-incubated with a single interferent under the same conditions as described above; percent brightness (%Br) was quantified using ImageJ. For matrix evaluation, human serum was diluted 10× in PBS, spiked with IL-6 (50 pg·mL⁻¹–5 ng·mL⁻¹), and analyzed using the same procedure; recoveries and RSDs (*n* = 3) were subsequently calculated.

2.7. Image analysis

The optical textures of the LC films were observed using a polarized optical microscope (Eclipse LV100 POL; Nikon, Tokyo, Japan) equipped with a digital camera (DS-2Mv). The microscope was operated at 10× magnification, and all images were captured under identical illumination and exposure conditions.

To ensure robust and unbiased brightness quantification, the mean grayscale intensity was calculated over the entire area of each TEM grid supporting the LC film, rather than selected local regions. For each experimental condition, multiple independently prepared TEM grids were analyzed, and the reported %Br values represent the average across different grids. The LC film thickness was strictly defined by the thickness of the TEM grid, and excess LC material outside the grid area was removed using a capillary tube prior to sealing, thereby minimizing thickness-induced optical variations. All polarized optical microscopy images were acquired using fixed microscope parameters (polarizer/analyzer configuration, illumination intensity, and exposure time), and brightness analysis was performed directly on the raw images without any post-acquisition image processing or contrast adjustment.

Although LC films may exhibit local texture heterogeneity at the microscopic scale, ensemble-averaged brightness values obtained over the full grid area and across multiple grids yielded reproducible and monotonic optical responses as a function of IL-6 concentration, with relative standard deviations below 5%.

3. Results and discussion

3.1. Principle of the aptamer-LC sensing mechanism

The detection principle of the developed LC-based aptasensor is schematically illustrated in Fig. 1. The sensing mechanism integrates the molecular recognition of an IL-6 aptamer with interfacial alignment transitions of UV-functionalized nematic LCs.

Under UV irradiation, 5CB underwent selective photooxidation at its terminal alkyl chain to generate a carboxylated LC phase containing CBA-like mesogenic species, rather than a single well-defined compound. The introduced -COOH groups were subsequently activated via EDC/NHS coupling to covalently link an amino-terminated complementary oligonucleotide, consequently producing a CBA-complementary strand conjugate uniformly dispersed in the LC phase. This functionalized LC matrix served as a responsive medium capable of translating nucleic acid hybridization events into optical signals.

As shown in Fig. 1, the aptamer preferentially bound to its target through specific conformational folding and hydrogen bond interactions in the presence of IL-6. This binding sequesters the complementary tail and prevents its hybridization with the surface-anchored complementary strand, thereby minimizing interfacial perturbation. As a result, the homeotropic alignment of LC molecules was preserved, yielding a uniformly dark optical appearance. The surface-anchored complementary oligonucleotide was rationally designed by the authors to hybridize exclusively with an artificial tail sequence appended to the 3' end of the IL-6 aptamer, rather than the target-binding domain itself. Specifically, the IL-6 recognition sequence (CTT CCA ACG CTC GTA TTG TCA GTC TTT AGT) was extended with a poly(T/G/A)-rich tail (TTT TTT GGG GGA AAA A), which enables predictable and reversible hybridization with the amino-modified complementary strand (/5AmMC6/TTT CCC CCA AAA A-3') immobilized at the liquid crystal interface. This design strategy preserves the structural integrity and binding functionality of the aptamer toward IL-6, while allowing duplex formation to modulate interfacial free energy in the absence of the target. Upon IL-6 binding, the stronger aptamer-IL-6 interaction competitively disrupts the tail-mediated hybridization, leading to a detectable LC reorientation.

In the absence of IL-6, the single-stranded tail of the IL-6-specific aptamer hybridized with the immobilized complementary sequence within the LC phase. This hybridization increases interfacial charge density and hydration, thereby disturbing the balance of the interfacial free energy at the aqueous/LC boundary. The resulting elastic distortion of the LC director disrupts homeotropic anchoring, leading to a bright planar optical texture under crossed polarizers.

The optical transition between the bright and dark states originates from modulation of Rapini-Papoular-type anchoring energy at the OTS/LC interface, which competes with elastic deformation induced by surface-associated nucleic acids. Although nonspecific adsorption of nucleic acids or proteins at the aqueous/LC interface cannot be entirely excluded, multiple experimental observations indicate that a hybridization-mediated mechanism is the dominant contributor to the LC response. Structurally and functionally diverse proteins, even at equal or higher concentrations, did not induce comparable dark textures or brightness attenuation, and mixture experiments demonstrated that LC alignment remained unperturbed unless IL-6 was present. These results indicate that generic interfacial perturbations alone are insufficient to account for the observed optical switching, which is consistent with competitive disruption of aptamer-complementary strand hybridization upon IL-6 binding. Consequently, molecular recognition of IL-6 was transduced into a distinct and measurable optical response, forming the basis for label-free and real-time cytokine detection.

3.2. Characterization of CBA formation

Fourier transform infrared (FT-IR) analysis confirmed the UV-

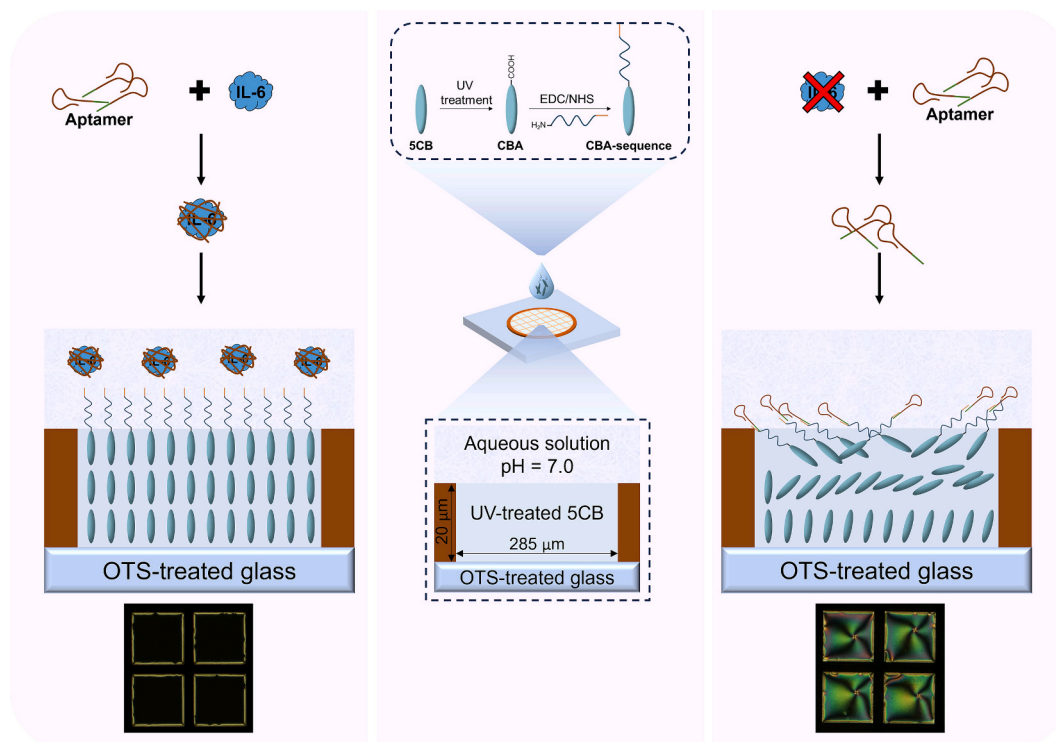


Fig. 1. Schematic illustration of the IL-6 detection mechanism based on UV-induced CBA-functionalized liquid crystals.

induced conversion of 5CB to CBA (Fig. 2A). Pristine 5CB exhibited a sharp $\nu(\text{C}\equiv\text{N})$ at $\sim 2225\text{--}2230\text{ cm}^{-1}$; intense $\nu(\text{C}\text{--}\text{H})$ bands of the pentyl chain at 2958, 2926, and 2855 cm^{-1} ; and aromatic framework bands at ~ 1605 , 1580, and 1500 cm^{-1} ; no carbonyl or acid O—H

features were observed [34]. After UV treatment, the spectrum gained a strong $\nu(\text{C}=\text{O})$ at $\sim 1710\text{--}1715\text{ cm}^{-1}$, a broad O—H band ($3300\text{--}2500\text{ cm}^{-1}$), and $\nu(\text{C}\text{--}\text{O})/\delta(\text{OH})$ features at $\sim 1310/1250\text{ cm}^{-1}$; these features are all indicative of a carboxylic acid. Concomitantly, the alkyl $\nu(\text{C}\text{--}\text{H})$ bands at $2958\text{--}2855\text{ cm}^{-1}$ markedly diminished/vanished, indicating oxidative removal of the pentyl chain. In contrast, the $\nu(\text{C}\equiv\text{N})$ near $\sim 2225\text{ cm}^{-1}$ and the aromatic modes persisted with minor changes in intensity. Collectively, these changes confirm the transformation of 5CB to CBA. This transformation replaced the terminal alkyl substituent with an --COOH group suitable for subsequent covalent coupling.

The conjugation of CBA with the amino-modified complementary oligonucleotide (/5AmMC6/TTTTTGGGGGAAAAA-3') was verified using surface and vibrational analyses. After the EDC/NHS activation of CBA to the NHS-ester, coupling with the terminal amine produced an amide linkage. The amide I and II bands appeared at $\sim 1655\text{--}1660\text{ cm}^{-1}$ and $\sim 1540\text{--}1555\text{ cm}^{-1}$, respectively, in the FT-IR spectra of the CBA-complementary oligonucleotide conjugate. These were accompanied by an amide III/ $\nu_{\text{as}}(\text{PO}_2^-)$ envelope at $\sim 1245\text{--}1280\text{ cm}^{-1}$ and $\nu_{\text{s}}(\text{PO}_2^-)$ at $\sim 1090\text{ cm}^{-1}$ from the oligonucleotide backbone. Concomitantly, the broad O—H stretch of the carboxylic acid ($3300\text{--}2500\text{ cm}^{-1}$) diminished markedly and the acid C=O band at $\sim 1710\text{ cm}^{-1}$ was replaced by the amide I feature, indicating consumption of --COOH groups during amide formation. The $\nu(\text{C}\equiv\text{N})$ of the biphenyl core persisted at $\sim 2225\text{--}2230\text{ cm}^{-1}$, whereas aliphatic $\nu(\text{C}\text{--}\text{H})$ at 2958/2926/2855 cm^{-1} increased slightly due to the AmMC6 linker.

This chemical modification was further supported by contact angle measurements, which showed a considerable change in the surface wettability of the CBA-functionalized film after oligonucleotide attachment. The contact angle decreased from $93.32 \pm 2.56^\circ$ for CBA to $66.72 \pm 3.81^\circ$ for the CBA-complementary oligonucleotide conjugate (Fig. 2B). This demonstrates the introduction of hydrophilic phosphate and amine moieties from the oligonucleotide. These changes indicate successful surface functionalization and improved interfacial compatibility between the complementary oligonucleotide and aqueous environment.

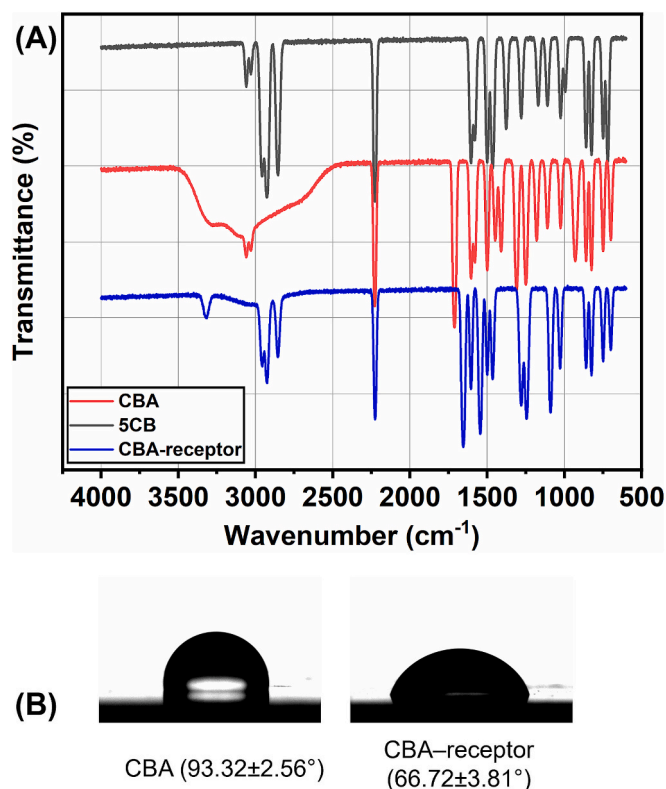


Fig. 2. (A) FTIR spectra of 5CB, CBA, and the CBA-receptor conjugate. (B) Water contact angles of CBA and the CBA-receptor film.

The spectroscopic and surface analyses confirmed stepwise fabrication of the complementary oligonucleotide-functionalized LC phase. The UV-induced oxidation of 5CB provided a chemically reactive mesogenic framework, whereas EDC/NHS coupling enabled stable anchoring of the complementary oligonucleotide via covalent amide linkages. This hybrid LC system retained the optical anisotropy of 5CB while incorporating a specific molecular recognition functionality, consequently providing a theoretical basis for the optical aptasensing of IL-6 based on interfacial alignment transitions.

3.3. Optimization of CBA doping concentration and aptamer concentration

CBA and aptamer concentrations were optimized to maximize optical contrast and reproducibility. The doping ratio of CBA in 5CB determines the density of the reactive sites and stability of LC alignment at the aqueous/LC interface. To identify the optimal concentration, a series of LC mixtures containing 0.1, 0.3, 0.5, 1.0, and 2.0 wt% CBA were prepared and tested under identical conditions using the same aptamer and complementary sequences (Fig. 3a). The optical texture under POM remained relatively bright at a low CBA content (<0.3 wt%), indicating incomplete functionalization and insufficient complementary oligonucleotide coverage to produce a stable anchoring transition. Increasing the concentration to 0.5 wt% resulted in a uniform dark homeotropic background and a distinct optical transition upon aptamer–complementary oligonucleotide interaction; this provided the highest signal-to-noise ratio. A further increase in CBA content (>1.0 wt%) caused aggregation and partial phase separation within the LC matrix, leading to non-uniform domains and decreased optical contrast. Therefore, 0.5 wt% CBA was selected as the optimal doping concentration to ensure both structural homogeneity and strong interfacial sensitivity for IL-6 detection. Batch-to-batch reproducibility of the UV-modified LC films was verified by repeated preparation under identical UV conditions, yielding comparable baseline brightness and relative standard deviations below 5%.

The concentration of the IL-6-specific aptamer determines the hybridization equilibrium and degree of interfacial perturbation. An excessively low concentration results in incomplete hybridization with the complementary strand, whereas excessive aptamer loading may cause non-specific adsorption or steric hindrance. To optimize this parameter, aptamer solutions with concentrations of 0.05, 0.1, 0.2, 0.5, and 1.0 μM were evaluated at a fixed IL-6 concentration of 1 $\text{ng}\cdot\text{mL}^{-1}$. At concentrations <0.1 μM , the LC films showed weak and unstable optical responses due to insufficient hybridization (Fig. 3b). A sharp and reproducible optical transition was observed between IL-6-free (bright planar) and IL-6-bound (dark homeotropic) states at 0.2 μM , producing

the maximum contrast in brightness. Higher aptamer concentrations (>0.5 μM) slightly reduced optical contrast, likely due to non-specific adsorption of excess aptamer molecules at the aqueous/LC interface (Fig. 3b). Thus, an aptamer concentration of 0.2 μM was determined to be optimal for reliable and quantitative IL-6 sensing.

3.4. Optical observation and quantitative analysis

The optical responses of the complementary oligonucleotide-functionalized LC films were investigated using POM to evaluate their sensing behavior toward IL-6 and establish a quantitative relationship between the IL-6 concentration and optical output. Upon introduction of the CBA–complementary oligonucleotide conjugate into the aqueous interface containing the IL-6 aptamer, distinct optical transitions were observed depending on the presence or absence of IL-6. The unbound aptamer hybridized with the immobilized complementary oligonucleotide in the absence of IL-6. This increased the interfacial charge density and disturbed the vertical anchoring of the LC molecules. This disruption induced a planar or tilted orientation, resulting in a bright optical texture under crossed polarizers. Conversely, the aptamer selectively bound to its target through hydrogen bonding and electrostatic interactions in the presence of IL-6, thereby preventing the hybridization of its tail region with the complementary strand. The interfacial disturbance was minimized and the LC molecules retained their homeotropic alignment, consequently producing a dark optical image. Consistent with this mechanism, Fig. 4a shows a monotonic attenuation of image brightness as [IL-6] increases from 0 $\text{pg}\cdot\text{mL}^{-1}$ to 10 $\text{ng}\cdot\text{mL}^{-1}$. LC domains evolved from bright, multicolor schlieren textures at 0 $\text{pg}\cdot\text{mL}^{-1}$ to progressively darker, nearly black fields at higher concentrations. Quantification of this effect as %Br yielded a decreasing trend with $\log[\text{IL-6}]$, enabling straightforward calibration. These optical transitions—bright in the absence of IL-6 and dark in the presence of IL-6—enabled the direct, label-free visual detection of the analyte.

For quantitative analysis, the calibration curve was constructed by plotting %Br as a function of the logarithm of IL-6 concentration, yielding the linear relationship $y = -25.89x + 117.96$, where y represents %Br and x corresponds to $\log[\text{IL-6}]$. This semi-logarithmic calibration enables reliable back-calculation of unknown IL-6 concentrations from measured %Br values. Because logarithmic transformation is undefined at zero concentration, the blank sample (IL-6 = 0) was not included as a data point on the calibration plot but was used exclusively to determine baseline signal variability. Accordingly, the LOD was not derived directly from the slope of the log-transformed calibration; instead, in accordance with IUPAC recommendations, the LOD was defined as the IL-6 concentration corresponding to the mean blank response minus three times its standard deviation, projected onto

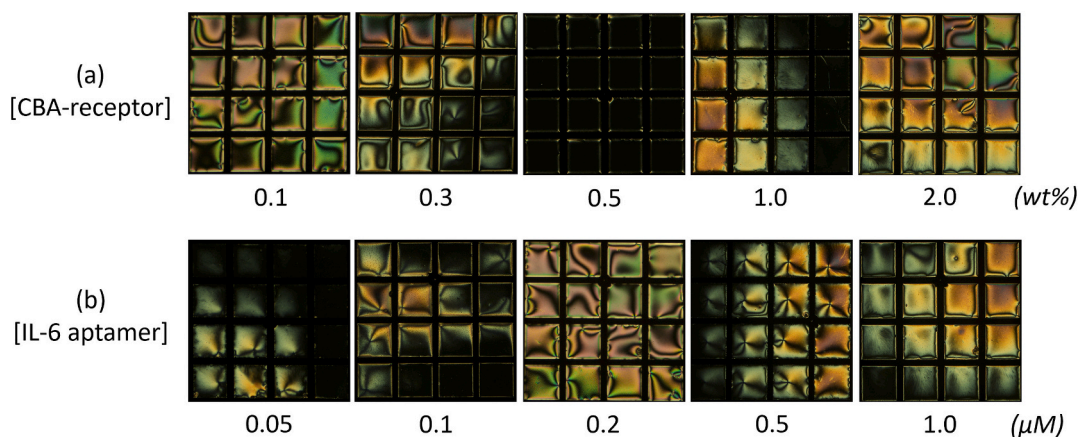


Fig. 3. Optical responses of (a) 5CB doping with CBA at concentrations of 0.1 to 2.0 wt%, and (b) CBA–receptor film at IL-6 aptamer concentrations of 0.05 to 1.0 μM . Mesh size = 285 μm .

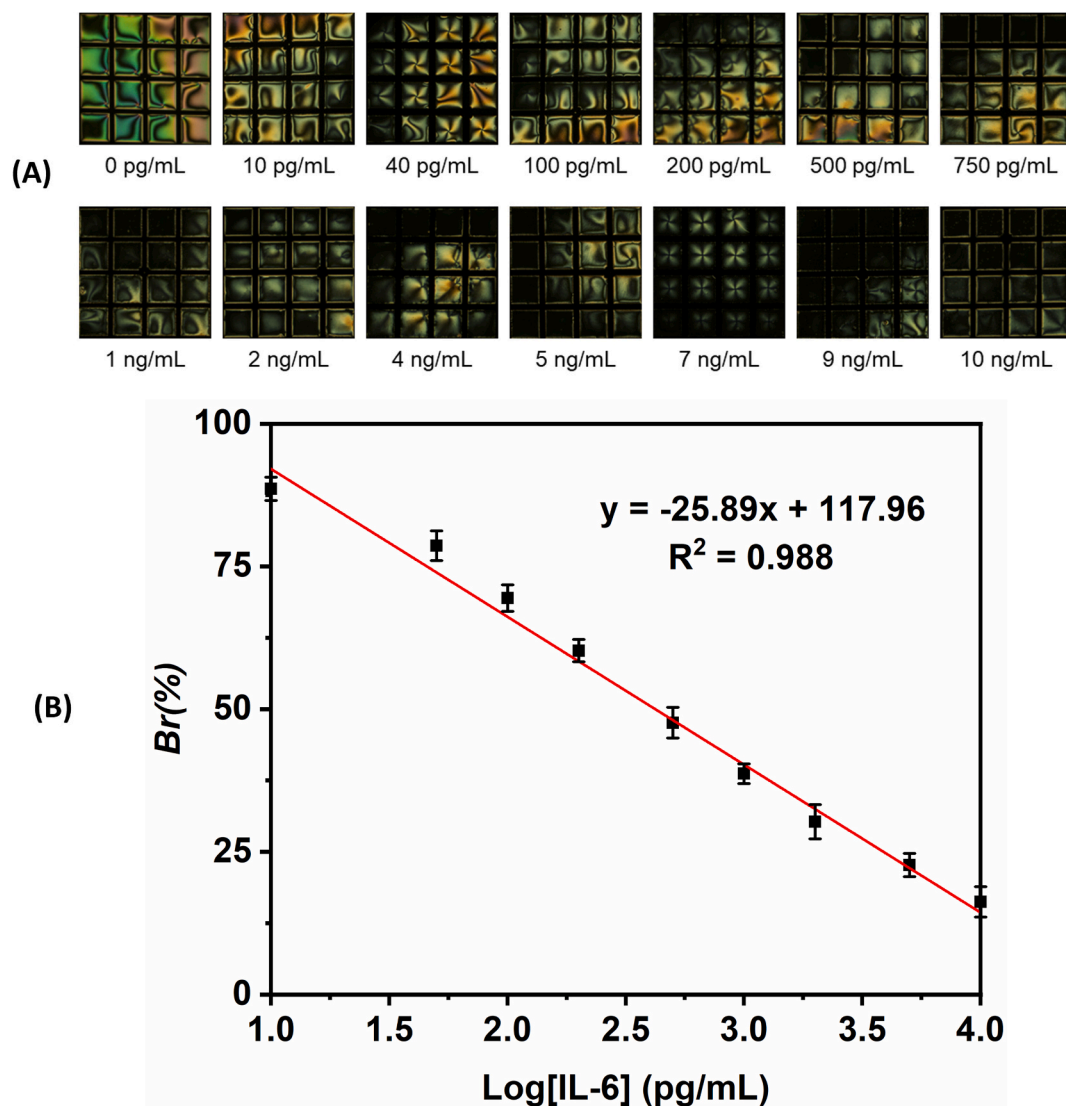


Fig. 4. (a) POM images of CBA–receptor doping LC films (TEM-grid/OTS-glass) for IL-6 = 10 pg·mL^{−1} to 10 ng·mL^{−1} (left→right), showing a monotonic decrease in brightness. (b) Calibration of %Br vs log₁₀[IL-6] with linear fit $y = -25.89x + 117.96$ ($R^2 = 0.988$); error bars = mean $\pm$ SD ($n = 3$). Mesh size = 285 μ m.

the semi-logarithmic calibration curve. Using this approach, the LOD was estimated to be 4.8 pg·mL^{−1}, indicating a high level of analytical sensitivity. The optical response stabilized within 30 min, confirming

rapid interfacial reorientation of the LC molecules. Furthermore, repeated measurements under identical conditions produced consistent brightness values with minimal variation (RSD < 5%), indicating the

Table 1

Comparison of materials, LODs, linear ranges, and samples used in previously reported methods for IL-6 detection.

Method	Material	LOD* (pg/mL)	Linear range (pg/mL)	Sample	Ref.
Electrochemical immunosensor	Interdigitated electrode array with antibody capture	1.63	10–10,000	Human CSF, human serum	[35]
Electrochemical immunosensor	Screen-printed electrode, Ab immobilization	0.78	2–250	Human serum	[36]
SERS immunoassay	Au nanostructures SERS substrate	0.028	0.1–1000	Human serum	[13]
LSPR imaging immunoassay	Nanoplasmonic chip, label-free	4.6	4.6–106	Human serum	[37]
Chromatic digital nanoplasmonometry	Self-referenced spectral LSPR chip	0.019	0.087–	Blood plasma	[38]
Electrochemiluminescence	Paired-signal ECL immunoassay	0.123	–	Clinical matrices	[39]
Molecularly imprinted polymer (MIP) electrochemical sensor	Poly(<i>o</i> -phenylenediamine) MIP on electrode	1.74	2–400	Human serum	[40]
Dual-target electrochemical aptasensor	Aptamers for IL-6 & TNF- γ on electrochemical platform	1.6	5–5000	Saliva and sweat	[41]
Liquid crystal aptasensor	UV-induced CBA–complementary oligonucleotide conjugate	4.8	10–10,000	Human serum**	This work

* All LOD and linear range values reported in Table 1 were determined in aqueous buffer unless otherwise specified.

** For this work, the LOD and linear range were evaluated in aqueous buffer. The corresponding original-sample-equivalent LOD in tenfold diluted human serum is approximately ten times higher.

excellent reproducibility and signal stability of the LC films.

The analytical performance of the developed LC-based aptasensor was compared with those of previously reported IL-6 detection methods (Table 1). Contrary to previously reported IL-6 assays, the LC-aptamer platform combines the specificity of aptamer recognition with the optical amplifying capability of LCs. This platform achieved comparable or superior sensitivity while drastically simplifying the detection procedure.

3.5. Selectivity of aptasensor for IL-6 detection

The selectivity of the LC aptasensor toward IL-6 was evaluated against common interferents (L-Lys, IL-4, IL-5, IFN- γ , Hgb, CEA, MUC1, MUC4, MUC16, and IgB; each 10 ng·mL⁻¹ in PBS), under the same protocol as that for IL-6 (Section 2.5). As anticipated based on the detection principle (Section 3.1 and Fig. 4a), targets that do not bind the IL-6 aptamer leave the interfacial hybridization intact, perturbing the LC to planar/tilted states and consequently maintaining a high percent brightness.

Fig. 5 (bar chart) summarizes the responses. All nontarget biomolecules yielded high %Br values and bright schlieren textures in the embedded POM thumbnails. In sharp contrast, IL-6 (10 ng·mL⁻¹) produced a markedly lower %Br with uniformly dark, homeotropic textures, consistent with aptamer–target binding that suppresses tail–complementary oligonucleotide hybridization. Mixture tests further validated the selectivity; a cocktail of interferents without IL-6 retained a high %Br comparable to that of the blank, whereas the same mixture spiked with IL-6 reproduced the low %Br observed for IL-6 alone. Across triplicates ($n = 3$), the %Br for IL-6 was significantly lower than that for every non-target, indicating negligible cross-reactivity, even in complex backgrounds.

Together with the monotonic brightness attenuation versus [IL-6] in Fig. 4a and the linear calibration of %Br vs. log[IL-6] in Fig. 4b, these data confirm that the aptamer–LC interface provides high selectivity for IL-6 and reliable discrimination against structurally and functionally related biomolecules, rather than implying absolute exclusivity.

3.6. Real-sample analysis

Real-sample measurements were performed using tenfold diluted human serum as a representative biological matrix to evaluate the practical applicability of the developed LC-based aptasensor. Human serum presents a stringent test because of its abundance of proteins,

lipids, and salts that can screen for charges or foul interfaces. A tenfold dilution with PBS was therefore applied as a simple pretreatment step to reduce matrix effects and viscosity while maintaining IL-6 concentrations within the sensor's dynamic range. Known concentrations of IL-6 standard solutions (50 pg·mL⁻¹, 500 pg·mL⁻¹, 1 ng·mL⁻¹, and 5 ng·mL⁻¹) were spiked into the diluted serum to prepare test samples. The calculated recovery rates ranged from 97.22% to 100.65%, and the RSD values were all below 5%, confirming good precision and repeatability (Table 2). No obvious interference or false responses were observed under these conditions, suggesting that the LC interface exhibits satisfactory resistance to nonspecific adsorption after serum dilution. This demonstrates the excellent antifouling characteristics of the LC interface.

These results demonstrate that the proposed LC-based aptasensor is capable of sensitively and accurately quantifying IL-6 in tenfold diluted human serum following a simple dilution step, without the need for labeling. It should be emphasized that the reported sensing performance reflects diluted-serum conditions, and antifouling performance in undiluted serum has not been explicitly demonstrated in the present study. While serum dilution reduces matrix complexity and does not fully represent undiluted clinical samples, this approach is commonly adopted to mitigate nonspecific interfacial effects in LC-based sensing systems and supports the bioanalytical feasibility of the proposed platform rather than immediate clinical readiness.

3.7. Stability of the UV-modified LC aptasensor

The storage stability of the UV-modified LC aptasensor was systematically evaluated to assess its practical feasibility. The functionalized LC films were stored under two different conditions: at room temperature (25 °C) and under refrigeration (4 °C). At predetermined time intervals, the baseline brightness (%Br) and IL-6 sensing response were recorded under identical experimental conditions.

When stored at room temperature (25 °C), the UV-modified LC films maintained stable optical responses and baseline brightness for up to 10 days, with no significant degradation in signal intensity or sensing performance. In contrast, storage at 4 °C further prolonged the stability, with consistent optical responses observed for at least 20 days (Fig. S1). These results indicate that the UV-modified LC interface and the immobilized complementary oligonucleotide remain chemically and optically stable over extended periods under appropriate storage conditions. The observed stability supports the reproducibility and practical feasibility of the proposed LC-based aptasensing platform, although further long-term and operational stability studies may be required for specific application scenarios.

4. Conclusion

This study introduces a label-free LC aptasensor based on an integrated aptamer–complementary oligonucleotide–LC platform that converts molecular recognition into optical signals. The UV-induced oxidation of 5CB to CBA, and the subsequent EDC/NHS coupling enabled covalent complementary oligonucleotide anchoring within the LC phase to provide a responsive interface for IL-6 detection. Aptamer–

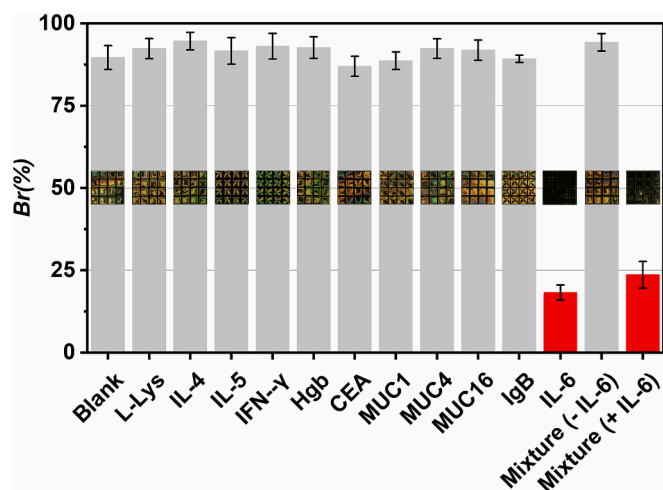


Fig. 5. A bar plot of percentage brightness (%Br) for non-target biomolecules (L-Lys, IL-4, IL-5, IFN- γ , Hgb, CEA, MUC1, MUC4, MUC16, and IgB; each 10 ng·mL⁻¹ in PBS) compared with that for IL-6.

Table 2

Recovery and precision of IL-6 detection in human serum samples.

Amount added (pg·mL ⁻¹)	Amount detected (pg·mL ⁻¹)		Recovery (% $n = 3$)	RSD (%)
	ELISA kit	LC-sensor		
0	Non-detectable	Non-detectable	–	–
50	50.03	48.61	97.22	3.54
500	499.87	503.24	100.65	2.87
1000	1001.66	995.65	99.57	4.23
5000	5003.29	4992.68	99.85	4.58

complementary strand hybridization served as a molecular switch for LC alignment transitions. The sensor exhibited a linear response over the range of $10 \text{ pg}\cdot\text{mL}^{-1}$ to $10 \text{ ng}\cdot\text{mL}^{-1}$, a detection limit of $4.8 \text{ pg}\cdot\text{mL}^{-1}$, and good reproducibility ($\text{RSD} < 5\%$), with high selectivity and accurate recovery (97.22–100.65%) demonstrated in tenfold diluted human serum. While the present sensing configuration relies on laboratory-based polarized optical microscopy for signal readout, this approach was primarily employed to validate the sensing mechanism under controlled conditions, and future work may focus on simplifying the optical readout toward more portable formats. Overall, these results highlight the potential of the proposed platform for LC-based bio-analytical sensing applications.

CRediT authorship contribution statement

Song Thai Duong Duong: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Chang-Hyun Jang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Compliance with ethical standards

The authors declare no potential conflicts of interest related to the publication of this article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.microc.2026.117168>.

Data availability

Data will be made available on request.

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