



Sensitive detection of florfenicol residues in food matrices using a label-free liquid crystal aptasensor with UV-functionalized 4-cyano-4'-biphenylcarboxylic acid

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Abstract

A label-free liquid-crystal aptasensor is reported that transduces florfenicol (FFC)–aptamer binding into optical responses. Nematic 4'-pentyl-4-biphenylcarbonitrile was UV-oxidized to 4-cyano-4'-biphenylcarboxylic acid, whose carboxyl groups enabled coupling of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride/N-hydroxysuccinimide to an amino-terminated complementary oligonucleotide dispersed in the liquid-crystal phase. In FFC-free samples, the aptamer tail hybridized with the immobilized receptor, disrupting homeotropic anchoring and yielding a bright planar texture, whereas FFC binding suppressed hybridization, preserving a dark texture. The percentage brightness values derived from polarized optical microscope images decreased linearly with FFC level within 0.2–80 ng mL⁻¹, with a limit of detection of 0.12 ng mL⁻¹. The sensor also showed good discrimination against co-residual veterinary drugs and metabolites relevant to food matrices. Sensor applicability to real matrices was quantified using QuEChERS extracts of milk and eggs diluted 10× with phosphate-buffered saline and spiked with FFC; the sensor achieved recovery levels within the validation limits and equivalent results to those of high-performance liquid chromatography. The proposed aptasensor stabilizes within ~20 min, requires no labels or complex instrumentation, and can be readily adapted to other small-molecule residues by swapping the aptamer.

Keywords Liquid-crystal biosensor · Optical microscope imaging · Aptamer · Florfenicol detection · Label-free optical sensing · Food safety monitoring

Introduction

Florfenicol (FFC) is a third-generation amphenicol used extensively in livestock and aquaculture to treat gram-positive and gram-negative infections [1]. Its broad-spectrum efficacy, oral/parenteral availability, and relative hematotoxic safety compared to chloramphenicol have led to the widespread prophylactic and therapeutic use of FFC in food-producing animals [2, 3]. However, misuse and

extra-label administration can leave residues in edible tissues, milk, eggs, and aquatic products, raising concerns regarding consumer exposure and the development of antimicrobial resistance. Accordingly, maximum residue limits for FFC (often regulated together with its primary marker metabolite, florfenicol amine) have been established across jurisdictions and are routinely enforced through surveillance programs [4]. These programs require analytical methods capable of trace-level quantification in chemically complex matrices [5, 6].

Instrumental chromatographic techniques such as high-performance liquid chromatography (HPLC) with ultraviolet (UV)–visible and fluorescence detectors and liquid chromatography–tandem mass spectrometry [7] remain the standard detection approaches because of their high specificity and accuracy. However, these methods require labor-intensive extraction/clean-up, expensive instrumentation, and trained personnel, limiting their suitability for

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high-frequency, near-real-time screening. Immunoassays (ELISA/FLISA) [8, 9] and Raman-based strategies (SERS, tag-assisted SERS) [10–12] exhibit improved throughput and sensitivity but often depend on labeled reagents, multistep amplification, or engineered nanostructures that complicate fabrication and may reduce robustness in routine food testing. Thus, researchers continue to explore label-free, rapid, and field-deployable alternatives that maintain analytical rigor while simplifying operation.

Liquid crystals (LCs) offer a distinct transduction modality for label-free biosensing. Nematic mesogens such as 4-cyano-4'-pentylbiphenyl (5CB) exhibit long-range orientational order and strong birefringence; subtle variations in interfacial chemistry at the aqueous/LC boundary can reconfigure surface anchoring from homeotropic (optically dark under crossed polarizers) to planar/tilted (bright), or vice versa [13]. This interfacial sensitivity amplifies the molecular recognition events into macroscopic optical readouts that are readily captured by polarized optical microscopy (POM). When LCs are confined within microdomains (e.g., transmission electron microscopy (TEM) grid apertures) [14], hundreds of independent sensing sites can be patterned per slide, enabling statistically well-supported readouts and potential multiplexing without the need for labels or enzymatic substrates.

Aptamers are short single-stranded nucleic acids isolated by *in vitro* selection that recognize targets via sequence-encoded folding into defined secondary/tertiary motifs [15–17]. For small molecules, binding is typically mediated by shape complementarity and a network of hydrogen bonding, π - π /CH- π , and electrostatic interactions within compact pockets [18]. Because selection occurs under controllable buffer conditions, aptamers can be tailored to operate at ionic strengths and pH values relevant to the analytical workflow. When applied to antibodies, DNA aptamers offer several practical advantages for sensor engineering: (i) fully chemical synthesis with exceptional lot-to-lot reproducibility; (ii) straightforward terminal functionalization (amines, thiols, azides, fluorophores, spacers) that enables modular immobilization and assay re-configuration; (iii) robustness to temperature and organic cosolvents within defined limits; (iv) reversible target binding permitting regeneration; and (v) low cost and short lead times for redesign [19–21].

In this study, an interfacial LC transduction framework is adapted to achieve label-free FFC detection using an aptamer-governed hybridization switch. The nematic LC 5CB is photo-oxidized to 4-cyano-4'-biphenylcarboxylic acid (CBA) [14, 22], thereby introducing carboxyl groups that enable covalent linkage of an amino-terminated “receptor” oligonucleotide at the aqueous/LC interface. An FFC-binding aptamer engineered with a short tail complementary to the immobilized receptor serves as the molecular gate. In

FFC-free samples, receptor–aptamer hybridization perturbs the interfacial free energy and drives a planar LC texture (bright under POM). Conversely, in the presence of FFC, aptamer–target complexation in solution prevents hybridization and preserves homeotropic anchoring and a dark texture. Aptasensor performance is quantified using the percentage brightness derived from POM images across LC microdomains, which reveals a monotonic response to FFC concentration.

By coupling sequence-specific molecular recognition with the intrinsic interfacial sensitivity of nematic LCs, the proposed platform provides a visual, real-time readout without labels or enzymatic substrates and requiring only small reagent volumes. The specific objectives of this study are as follows: (i) construct a CBA-functionalized LC interface bearing a receptor strand compatible with the FFC aptamer; (ii) optimize key variables governing anchoring transitions (aptamer/receptor stoichiometry, ionic strength, incubation time, and imaging parameters); (iii) determine key analytical values, including the limit of detection, linear range, precision, and stability; (iv) evaluate sensor specificity against structurally related phenicols and common co-residuals; and (v) validate sensor accuracy and recovery in representative food matrices.

Materials and methods

Materials and reagents

The nematic LC 5CB was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Glass microscope slides (76 × 26 mm, 1.0 mm thick) were obtained from Matsunami Glass Ind., Ltd. (Osaka, Japan). Copper TEM grids (75 mesh; pitch 340 μ m; hole 285 μ m; bar 55 μ m) were purchased from Gilder Grids (Grantham, UK). Sulfuric acid (H_2SO_4 , $\geq 95\%$), hydrogen peroxide (H_2O_2 , 30%), methylene chloride (CH_2Cl_2 , $\geq 99.5\%$), octyltrichlorosilane (OTS, $\geq 97\%$), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were obtained from Daejung Chemicals & Metals Co., Ltd. (Siheung, Korea). Ultrapure deionized water (18.2 $\text{M}\Omega\cdot\text{cm}$) was produced using a Milli-Q system (Millipore, Billerica, MA, USA).

Florfenicol (FFC, analytical standard), chloramphenicol (CAP), thiamphenicol (TAP), florfenicol amine (FFA), tetracycline (TC), oxytetracycline (OTC), enrofloxacin (ENR), amoxicillin (AMO), methicillin (MET), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (LC-MS grade), ethanol ($\geq 99.8\%$), sodium chloride (NaCl), magnesium sulfate (MgSO_4 , anhydrous),

and sodium acetate (NaOAc), which were used to derive extracts according to the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

The amino-modified complementary receptor strand ****/5AmMC6/**TTT TTCC CCC AAA AA-3'**, the FFC-binding DNA aptamer **5'- GCT GTG TGA CTC CTG CAA GGT CCA TTC AAG TCG TAG GTT TGC CTT CAG CCT CAA CGC TTA CGC AGC TGT ATC TTG TCT CCT TTT TGG GGG AAA AA -3'** (HPLC-purified) [23], and a random-sequence aptamer (**5'-ATT GCA CCT ACT GTC GTA ACT AGT CCA TTC GGC AGT CTG AGG AAT GGC AAG CCA GGT AAG TTC CCA GAG CTC AAG CCA GGT TTT TGG GGG AAA AA-3'**) were synthesized by Integrated DNA Technologies (IDT, Singapore). Phosphate-buffered saline (PBS; 10 mM phosphate, 137 mM NaCl, 2.7 mM KCl; pH 7.4) was purchased from Thermo Fisher Scientific. Unless otherwise stated, all reagents were used as received without further purification.

Preparation of OTS-modified glass substrates

To generate a high-density layer of surface –OH groups, glass slides were immersed in freshly prepared piranha solution (H₂SO₄ 95%: H₂O₂ 30% = 7:3, v/v) at 80 °C for 60 min, rinsed copiously with DI water ($\geq 5\times$), followed by rinsing with ethanol, then dried with nitrogen. The slides were baked at 120 °C for 1 h to remove adsorbed moisture [24].

Silanization was performed in anhydrous heptane containing 0.2% (v/v) OTS (25 °C, 30 min). Slides were rinsed with heptane, CH₂Cl₂, and EtOH, dried under N₂, and cured at 120 °C (1 h). The substrates were then stored in a desiccator for <2 weeks.

UV oxidation of 5CB to CBA

5CB was UV-oxidized using a long-wavelength protocol. Briefly, 600 μ L of neat 5CB was dispensed into a clean, open-mouth 10-mL borosilicate vial and positioned 8 cm beneath a Spectroline EN-280 L long-wave UV lamp (365 nm) equipped with two 8-W tubes and a filter assembly. Irradiation was performed for 48 h in a dark room to eliminate ambient light. Active stirring was not used; instead, the vials were gently rotated every 12 h to ensure uniform exposure. After two days, the material developed a faint yellow hue, indicating successful photo-oxidation [22].

Fabrication of the CBA-functionalized LC receptor

To prepare the CBA-functionalized LC receptor, CBA (10 mg) was dissolved in 1.0 mL of an ethanol/water mixture

(1:1, v/v). EDC and NHS were added at final concentrations of 10 and 25 mM, respectively, followed by gentle stirring at room temperature for 30 min to activate the carboxyl groups of CBA. Subsequently, the amino-terminated complementary oligonucleotide (**/5AmMC6/TTT TTT GGG GGA AAA A-3'**) was added to a final concentration of 1.0 μ M, and the mixture was incubated for 2 h at room temperature to allow covalent amide-bond formation between activated CBA and the oligonucleotide. After the coupling reaction, the solution containing the CBA–oligonucleotide conjugate was purified to remove unreacted CBA and residual activating reagents. The reaction mixture was filtered, diluted with PBS (10 mM, pH 7.4), and centrifuged. This washing step was repeated three times. The retentate containing the purified CBA–oligonucleotide conjugate was collected and lyophilized to obtain a dry solid.

For LC receptor preparation, the dried CBA–oligonucleotide conjugate was mixed with 5CB at 40–45 °C to obtain a homogeneous LC phase containing 0.5 wt% of the conjugate. The mixture was vortexed and sonicated briefly until uniform. The resulting CBA-functionalized LC phase was used as the receptor phase in all subsequent experiments.

Detection procedure

FFC was detected using receptor-functionalized CBA films supported on copper TEM grids. The workflow comprised three stages: (i) pre-incubation of the FFC aptamer with the analyte; (ii) application of the sample to the aqueous/LC interface; and (iii) optical observation and quantitative analysis of the LC response.

Pre-incubation (solution-phase recognition)

The FFC aptamer was thermally folded by heating to 95 °C for 5 min, snap-cooled on ice for 5 min, then equilibrated at room temperature (10 min). The aptamer was diluted to a working concentration (0.2 μ M) in PBS. Defined volumes of FFC standards or diluted matrix extracts were added to the aptamer solution to achieve final analyte concentrations spanning 0.01–100 ng·mL^{–1}. The mixture was gently mixed and incubated at room temperature for 10 min to allow for specific complexation between FFC and the aptamer recognition domain.

Application to the aqueous/LC interface

Receptor-functionalized CBA films were prepared within the openings of copper TEM grids placed on OTS-coated glass slides. Approximately 1 μ L of receptor-CBA/5CB mixture was introduced into each grid opening, and excess LC was removed with a microcapillary to obtain uniform

films. The LC-filled grids were then immersed in 200 μL of the pre-incubated aptamer–sample solution, ensuring complete and quiescent contact between the aqueous phase and LC interface. The assembly was left undisturbed for 10–15 min to permit interfacial hybridization (FFC-absent) or suppression by FFC–aptamer complexation (FFC-present), thereby modulating LC anchoring.

Selectivity and real sample testing

Selectivity protocol

Interference was evaluated using a defined cocktail of residual veterinary drugs and metabolites relevant to food matrices. The interferent set comprised CAP, TAP, FFA, TC, OTC, ENR, AMO, and MET, with each sample being prepared as 80 $\text{ng}\cdot\text{mL}^{-1}$ in PBS.

Food sample preparation

Food matrices were extracted according to the following QuEChERS-type procedure [7]: 2.0 g homogenized sample + 4.0 mL acetonitrile, vortex for 1 min; add 2.0 g MgSO_4 and 0.5 g NaCl, vortex for 1 min; centrifuge at $5000 \times g$ for 5 min. An aliquot (1.0 mL) of the acetonitrile layer was subjected to dispersive clean-up (25 mg PSA + 25 mg C18, vortexed for 30 s, and centrifuged at $5000 \times g$ for 3 min). The supernatant was evaporated under N_2 at 35 $^\circ\text{C}$ and reconstituted in PBS. The extracts were diluted 10-fold in PBS prior to mixing at a 1:1 ratio with the aptamer solution described above. For recovery experiments, extracts were spiked with FFC at concentrations of 5, 10, 20, 40 $\text{ng}\cdot\text{mL}^{-1}$ immediately before mixing with the aptamer. The spiking levels were selected to (i) lie well within the validated linear range of the LC-based aptasensor (0.2–80 $\text{ng}\cdot\text{mL}^{-1}$) and (ii) cover low-to-moderate concentrations relevant for assessing recovery and accuracy in complex food matrices. These levels were therefore used to evaluate method performance across the lower and mid portions of the working range, where quantitative discrimination is critical for residue screening.

Image analysis

Each copper TEM grid contained 32 intact square holes and approximately 20 non-square/partial holes at the periphery. Only the 32 intact square holes were used for quantification to ensure a consistent optical path length and ROI geometry. For each FFC concentration, 32 independent LC domains (square holes) were analyzed per chip, and the experiment was repeated using $k=3$ independently prepared sensor chips (independent receptor-phase preparation

and LC assembly). Thus, the total number of analyzed LC domains was $N=96$ per concentration, and data are reported as mean $\pm$ SD.

The optical textures of LC films were observed using a polarized optical microscope (Eclipse LV100 POL; Nikon, Tokyo, Japan) equipped with a digital camera (DS-2Mv). POM images were acquired under fixed microscope settings and processed in ImageJ as follows: (i) import and convert to 8-bit grayscale; (ii) define an ROI for each intact square hole using a consistent ROI size/shape; (iii) apply a fixed intensity threshold (50–170) to segment bright regions within each ROI; (iv) calculate %Br as the area fraction of threshold-selected pixels within the ROI; and (v) compute the chip-level mean by averaging the 32 ROI values, followed by calculation of the final mean $\pm$ SD from three independent chips for each concentration. Background correction was not applied because %Br was determined from within-ROI threshold segmentation using identical imaging conditions.

$$\%Br = \frac{A_{\text{bright}}}{A_{\text{ROI}}} \times 100$$

Where A_{bright} is the area of pixels with grayscale values within 50–170 and A_{ROI} is the total ROI area.

Results and discussion

Principle of the aptamer–LC sensing mechanism

The operating principle of the LC-based aptasensor is shown in Fig. 1. The sensing mechanism integrates small-molecule recognition by an FFC aptamer with the alignment transitions of a UV-functionalized nematic LC. Upon long-wave UV irradiation, 5CB undergoes selective photooxidation at its terminal alkyl chain to yield CBA. The introduced carboxyl ($-\text{COOH}$) groups are then activated via EDC/NHS chemistry to form covalent amide bonds with an amino-terminated complementary oligonucleotide, generating a CBA–receptor conjugate uniformly dispersed within the LC phase. This receptor-functionalized LC matrix transduces the nucleic acid hybridization events that occur at the aqueous/LC boundary into macroscopic optical signals.

As illustrated in Fig. 1, when FFC is present, the aptamer selectively binds its target via sequence-encoded folding and noncovalent interactions (hydrogen bonding, $\pi-\pi/\text{CH}-\pi$, and electrostatics). Target binding sequesters the short complementary tail of the aptamer, preventing hybridization with the immobilized receptor strand at the interface. The interfacial disturbance is thereby minimized, preserving homeotropic anchoring of the LC director and producing uniform dark domains under crossed-polarized light.

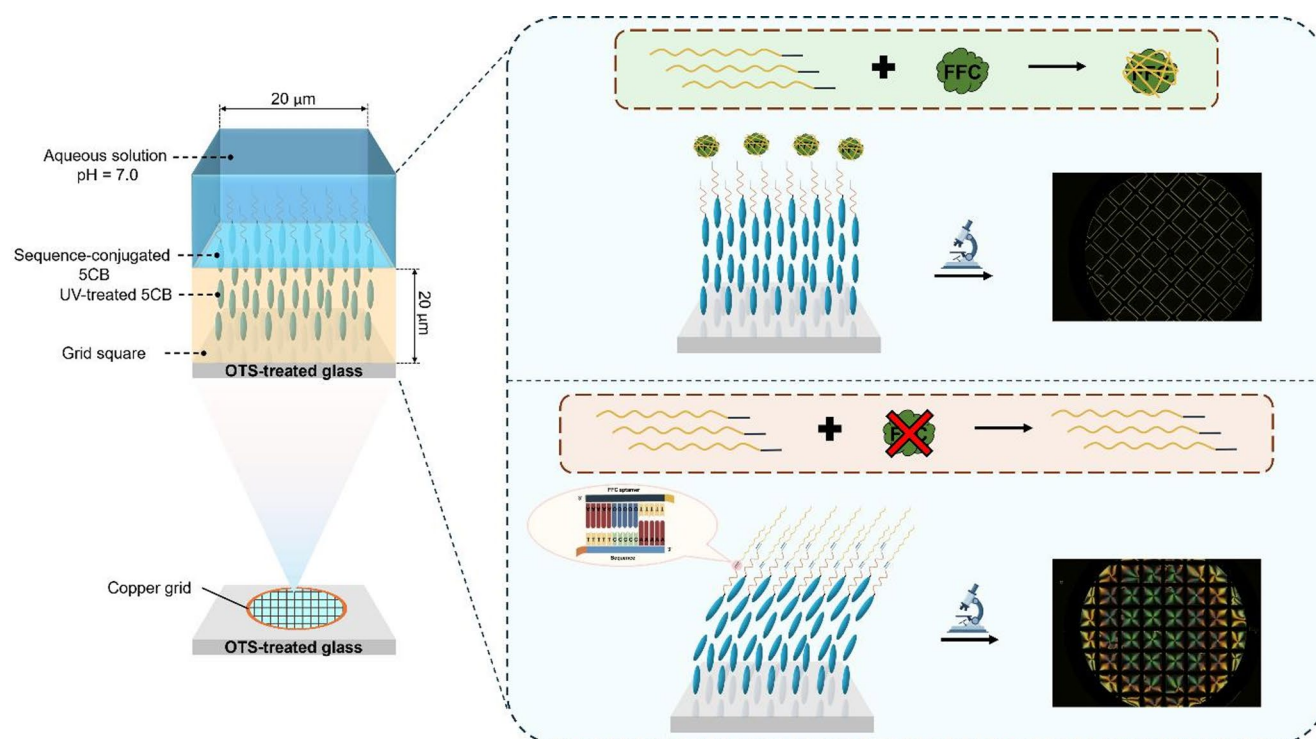


Fig. 1 Schematic and operating principle of the liquid crystal (LC)-based aptasensor for florfenicol. 5CB, 4-cyano-4'-pentylbiphenyl; OTS, octyltrichlorosilane

Conversely, in the absence of FFC, the single-stranded tail of the FFC-specific aptamer hybridizes with the receptor sequence at the LC interface. Duplex formation increases the interfacial charge density and hydration, alters the interfacial free energy, and introduces an elastic distortion in the LC director. Thus, the balance of surface anchoring shifts away from homeotropic alignment, yielding a bright planar texture. The bright-to-dark optical transition originates from the modulation of Rapini–Papoular-type surface anchoring energy at the OTS/LC interface, which competes with the elastic penalty induced by surface-adsorbed nucleic acids. FFC binding effectively restores the anchoring equilibrium toward the homeotropic state, producing a quantifiable decrease in the percentage brightness. Accordingly, the molecular recognition of FFC is transduced into a distinct, real-time, and label-free optical response suitable for quantitative analysis.

Characterization of CBA formation and functionalization

Fourier transform infra-red absorbance spectra confirmed the UV-induced conversion of 5CB to CBA and subsequent receptor conjugation (Fig. 2). Pristine 5CB exhibited a $\nu(\text{C}\equiv\text{N})$ band at $\sim 2225\text{--}2230\text{ cm}^{-1}$, alkyl $\nu(\text{C}\text{--}\text{H})$ bands at 2958, 2926, and 2855 cm^{-1} , and aromatic skeletal vibrations at ~ 1605 , 1580, and 1500 cm^{-1} , with no detectable carbonyl

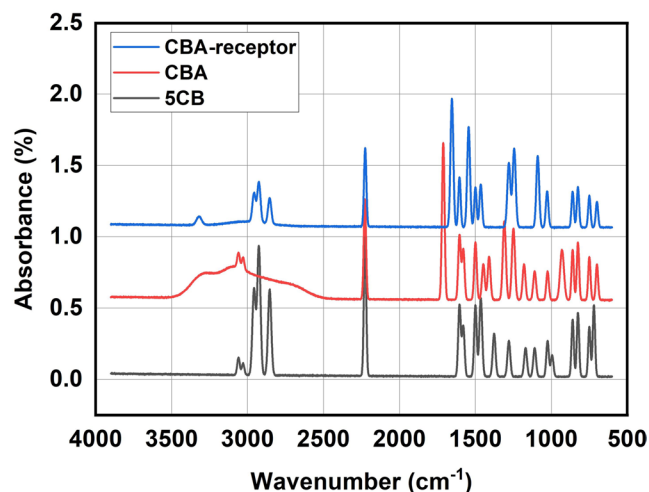


Fig. 2 Fourier transform infra-red characterization of 5CB, ultraviolet (UV)-oxidized 5CB, and receptor conjugation

or acid O–H features [25]. Long-wave UV irradiation led to the emergence of a strong $\nu(\text{C}=\text{O})$ band near 1710 cm^{-1} , a broad O–H envelope spanning 3300–2500 cm^{-1} , and characteristic $\nu(\text{C}\text{--}\text{O})/\delta(\text{OH})$ signals around 1310–1250 cm^{-1} , together with attenuation of the alkyl $\nu(\text{C}\text{--}\text{H})$ bands while retaining the $\nu(\text{C}\equiv\text{N})$ and aromatic modes; these changes indicated oxidative cleavage of the terminal pentyl chain and the formation of carboxylic acid groups suitable for coupling.

Conjugation of CBA with the amino-terminated complementary oligonucleotide following EDC/NHS activation was evidenced by the appearance of amide I (~ 1655 – 1660 cm^{-1}) and amide II (~ 1540 – 1555 cm^{-1}) bands, phosphate-associated bands of the DNA backbone ($\nu_{\text{as}}(\text{PO}_2^-)$ at ~ 1245 – 1280 cm^{-1} and $\nu_{\text{s}}(\text{PO}_2^-)$ near 1090 cm^{-1}), and a reduction of the broad acid O–H and carboxylic $\nu(\text{C}=\text{O})$ signals, which indicates the consumption of $-\text{COOH}$ groups during amide formation. A slight increase in aliphatic $\nu(\text{C}-\text{H})$ intensity was consistent with the AmMC6 linker, whereas the $\nu(\text{C}\equiv\text{N})$ band of the mesogenic core remained unchanged. Collectively, these spectral changes support the successful UV oxidation of 5CB to CBA and covalent attachment of the receptor strand within the LC matrix.

Optimization of CBA doping concentration and aptamer concentration

To enhance the optical contrast and analytical reproducibility, the mass fraction of CBA in 5CB was systematically evaluated while maintaining constant receptor/aptamer sequences and aptamer concentration (Fig. 3). Because CBA dictates the surface density of reactive sites and thereby influences the stability of LC anchoring at the aqueous/LC boundary, LC blends containing 0.1, 0.3, 0.5, 1.0, and 2.0 wt% CBA were prepared and examined under standardized conditions.

At $\leq 0.3\text{ wt\%}$ CBA, POM revealed persistently bright textures, indicative of incomplete functionalization and insufficient receptor coverage to elicit a stable anchoring transition (Fig. 3a–b). Increasing the load to 0.5 wt% yielded a uniform homeotropic baseline with a clear, reproducible brightness decrease upon aptamer–receptor gating, affording the highest signal-to-noise ratio and interdomain uniformity (Fig. 3c). Further elevation to $\geq 1.0\text{ wt\%}$ produced microstructural heterogeneity consistent with aggregation or incipient phase separation within the LC matrix, which diminished optical contrast and increased variability (Fig. 3d–e). Accordingly, 0.5 wt% CBA was selected as the working composition for subsequent FFC measurements

to balance the interfacial sensitivity with structural homogeneity.

The concentration of the FFC aptamer governs hybridization equilibrium with the immobilized receptor, and consequently the magnitude of the interfacial perturbation. Insufficient aptamer concentrations yield incomplete duplex formation, whereas excessive amounts can promote non-specific adsorption and steric congestion at the aqueous/LC boundary. To define the operating range, aptamer solutions of 0.05, 0.10, 0.20, 0.50, and $1.0\text{ }\mu\text{M}$ were evaluated at a fixed FFC level of $80\text{ ng}\cdot\text{mL}^{-1}$ under otherwise identical conditions (Fig. 4). At concentrations $< 0.10\text{ }\mu\text{M}$, LC films exhibited weak and unstable optical responses consistent with limited hybridization (Fig. 4a–b). An aptamer concentration of $0.20\text{ }\mu\text{M}$ produced a sharp, reproducible transition between the FFC-free (bright planar) and FFC-bound (dark homeotropic) states, affording the largest brightness contrast and lowest interdomain variability (Fig. 4c). Further increases to $\geq 0.50\text{ }\mu\text{M}$ modestly diminished optical contrast, likely owing to nonspecific interfacial adsorption of surplus aptamer and associated steric effects (Fig. 4d–e). Accordingly, $0.20\text{ }\mu\text{M}$ was adopted as the working concentration for subsequent quantitative analysis.

Optical observation and quantitative analysis

The optical behavior of the receptor-functionalized LC films was examined using crossed polarizers to establish a quantitative relationship between FFC concentration and image brightness. Introducing the CBA–receptor LC phase to an aqueous phase containing the FFC aptamer produced two distinct textures depending on the target availability. In the absence of FFC, the tail of the aptamer readily hybridized with the immobilized receptor, elevating interfacial charge/hydration and perturbing homeotropic anchoring; the LC adopted planar/tilted orientations and appeared bright. In the presence of FFC, solution-phase complexation sequestered the tail, suppressed interfacial hybridization, and minimized disturbance at the boundary, thereby preserving homeotropic alignment and yielding dark domains. Consistent with

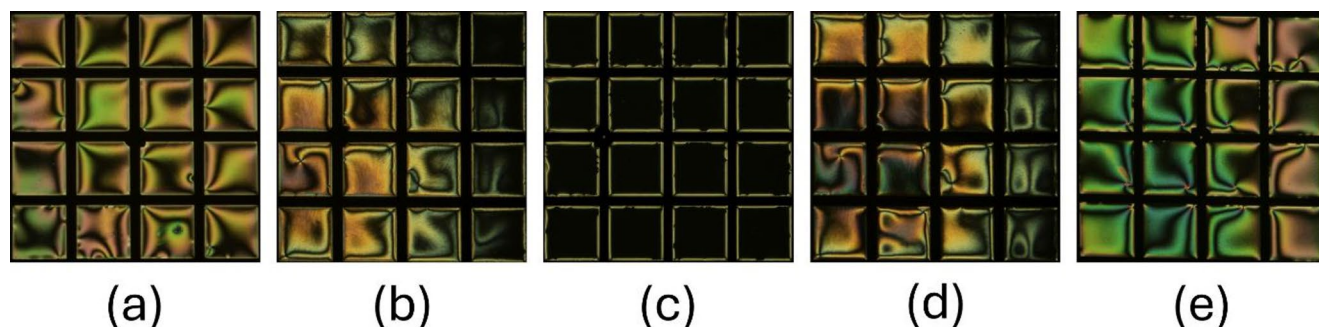


Fig. 3 Polarized optical microscopy (POM) images of LC films doped with (a) 0.1, (b) 0.3, (c) 0.5, (d) 1.0, and (e) 2.0 wt% 4-cyano-4'-biphenylcarboxylic acid (CBA). Mesh size = $285\text{ }\mu\text{m}$

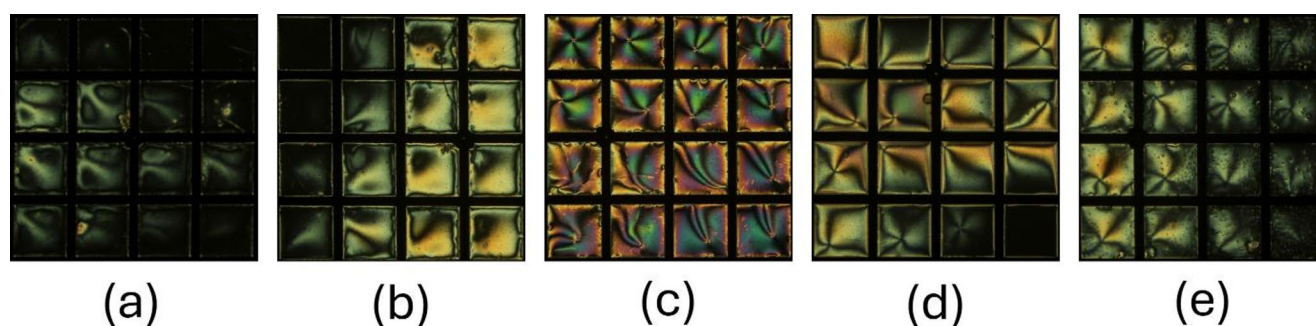


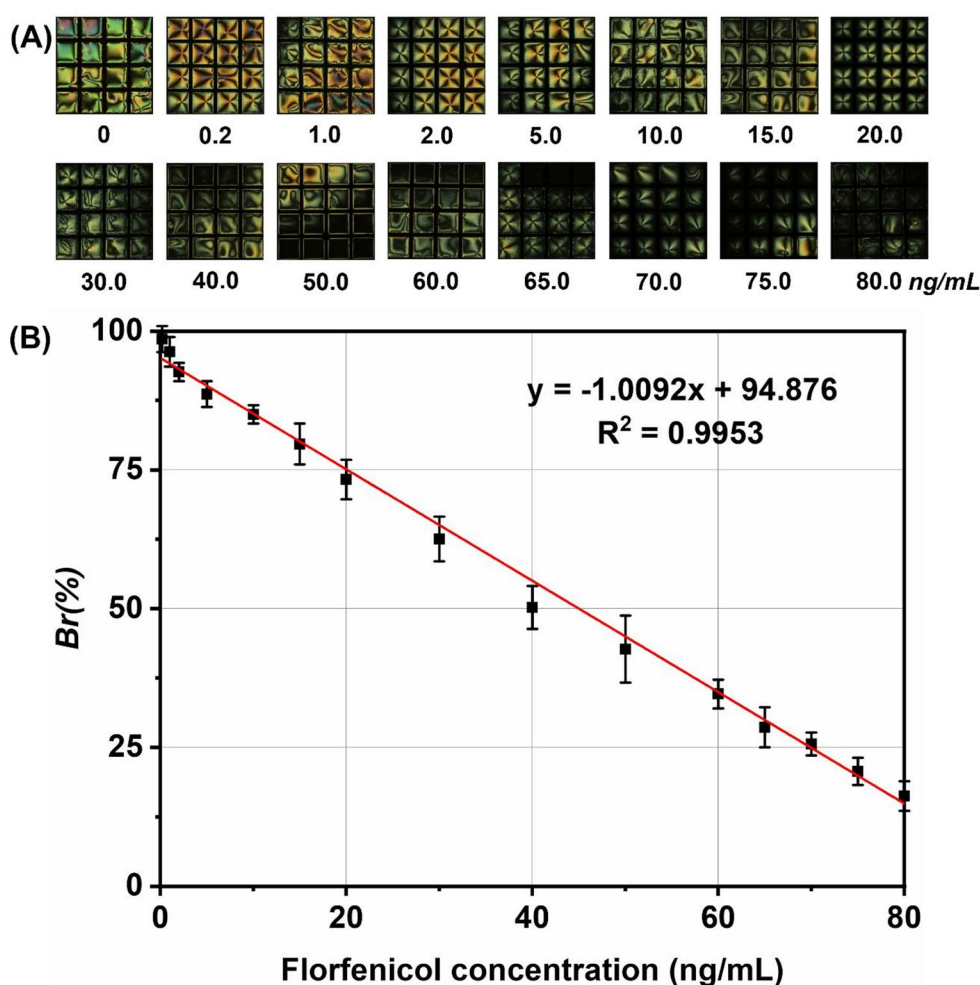
Fig. 4 POM images of receptor-functionalized LC films acquired under identical conditions at aptamer concentrations of (a) 0.05, (b) 0.10, (c) 0.20, (d) 0.50, and (e) 1.0 μM . Mesh size = 285 μm

this mechanism, Fig. 5A shows a monotonic attenuation of domain brightness with increasing FFC concentration (0–80 ng mL^{-1}), evolving from multicolor schlieren textures at low concentrations to almost black fields at high concentrations, enabling direct, label-free visualization of FFC.

For quantification, POM images were processed in ImageJ to obtain mean grayscale intensities for individual LC microdomains, which were converted to percentage brightness (%Br) values. For each FFC concentration, 32

intact square grid holes were analyzed per copper grid, and the measurement was repeated using three independently prepared sensor chips ($k=3$; total $N=96$ LC domains per concentration). Chip-level means (averaged from 32 holes) were used to calculate the final mean $\pm$ SD. %Br decreased linearly with FFC concentration over 0.2–80 ng mL^{-1} , following: $\%Br = -1.0092 \times [\text{FFC}] + 94.876$ (Fig. 5B). The limit of detection was estimated as $3\sigma/\text{slope}$, where σ is the standard deviation of the blank (based on 3 independent

Fig. 5 Optical response and calibration of the LC-based aptasensor for FFC. (A) Representative POM images of receptor-functionalized LC domains after exposure to FFC at concentrations from 0 to 80 ng mL^{-1} . (B) Calibration curve derived from ImageJ-based quantification, showing a linear decrease in percentage brightness (%Br) with increasing FFC concentration (0.2–80 ng mL^{-1}). For each concentration, 32 intact square grid holes were analyzed per chip, and measurements were performed on three independently prepared sensor chips ($k=3$). Error bars represent SD. Mesh size = 285 μm



chips), yielding an LOD of 0.12 ng mL^{-1} . Under the operating protocol (10 min pre-equilibration plus 10 min interfacial incubation), the signal approached a stable value within ~ 20 min. Collectively, these results indicate that interfacial LC reorientation enables repeatable and linear optical transduction of FFC under the tested conditions.

The analytical performance of the LC-based aptasensor for FFC was benchmarked against representative florfenicol assays reported in recent literature (Table 1). Notably, several reported approaches—particularly immunoassay- and SERS-based platforms—achieve wider linear ranges and/or lower LODs than the present assay. However, these gains are often accompanied by increased assay complexity, the use of signal tags (e.g., Raman labels, enzymes, or chemiluminescent/electroluminescent reporters), and reliance on specialized instrumentation. In contrast, while the sensitivity achieved here remains competitive for residue analysis, the primary advantage of the LC-based aptasensor lies in its operational simplicity and label-free workflow, enabling a direct optical (POM) readout with minimal reagents and instrumentation. Accordingly, this method is positioned as a practical screening/quantification tool that complements instrument-intensive techniques in settings where rapid operation and straightforward readout are prioritized.

Specificity and selectivity

Selectivity toward FFC was assessed using a standard protocol (Detection procedure) with a panel of co-residual antibiotics and the major FFC metabolite: CAP, TAP, FFA, TC, OTC, ENR, AMO, and MET (each 80 ng mL^{-1} in PBS). As expected from the detection mechanism, nontargets that do not bind to the FFC aptamer left the receptor–tail hybridization at the interface intact, thereby inducing planar/tilted alignment and maintaining a high percentage brightness value.

The optical responses are summarized in Fig. 6. All individual interferents produced high %Br with bright schlieren textures in the corresponding POM thumbnails, whereas FFC yielded markedly reduced %Br and uniformly dark, homeotropic textures, indicating that aptamer–target complexation disrupts the interfacial hybridization state. Mixture challenges further corroborated the specificity of the sensor: a cocktail containing CAP, TAP, FFA, TC, OTC, ENR, AMO, and MET (Mixture–FFC) yielded a %Br that was indistinguishable from the blank, whereas the same cocktail spiked with FFC (Mixture+FFC) yielded the lowest %Br observed. The %Br value obtained for FFC was significantly lower than those of all other veterinary drugs and matrix components ($p < 0.05$, $n = 3$), indicating that the LC-based aptasensor can selectively recognize FFC against potential interferents.

Table 1 Representative analytical methods for FFC screening in food matrices

Method	Material	Linear range (ng mL ⁻¹)	Limit of detection (ng mL ⁻¹)	Sample	Ref.
Magnetic relaxation switching + colorimetric dual-mode aptasensor	Aptamer-modified Au@Fe ₃ O ₄ nanocomposites	1.43–14.32 (MRS) 1.79×10^3 — 1.43×10^4 (colorimetric)	1.8	Egg extract	[26]
Time-resolved fluorescence immuno-chromatographic assay	Antibody (SF11)+Eu ³⁺ -doped fluorescent microspheres	0.42–8.40 in milk 0.83 to 9.83 in egg	0.23 in milk 0.48 in egg	Milk, egg	[1]
Gap-enhanced Raman tags competitive immunoassay	Au@4MBA@Au core-shell nanoparticles	0.1–1000	0.1	Milk	[27]
SERS paper sensor	3D ZnO@Ag nano-flowers on polyester fiber membrane	0.36– 0.36×10^6	0.07	Chicken meat	[11]
Quantum-dot fluorescence immunoassay	ZnCdSe/ZnS QDs-labeled antibody	0.6–30.4	0.3	Animal-derived foods	[28]
Flow injection chemiluminescence immunoassay	Ni/Co bimetallic MOF nanozymes	0.0001–1000	0.000033	Milk and chicken	[29]
Electrochemiluminescence immunoassay	ECL-RET from CeO ₂ @TiO ₂ to Cu ₂ S	0.001–1000	0.0003	Cow milk and fish tissue	[30]
Liquid crystal-based aptasensor	UV-induced CBA-receptor conjugate	0.2–80	0.12	Milk and egg	This work

To further exclude nonspecific effects of FFC and to confirm that the optical response is governed by specific target–aptamer complexation, a random-sequence aptamer control was introduced. In this control, the FFC aptamer was replaced with a random-sequence aptamer (5'-ATT GCA CCT ACT GTC GTA ACT AGT CCA TTC GGC AGT CTG

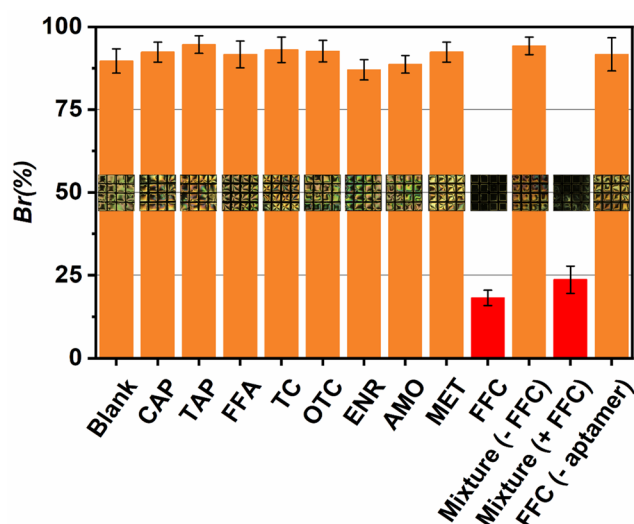


Fig. 6 %Br responses for the blank, individual interferents (CAP, TAP, FFA, TC, OTC, ENR, AMO, and MET; each 80 ng mL⁻¹), FFC (10 ng mL⁻¹), and mixtures of all interferents without (Mixture -FFC) or with FFC (Mixture +FFC). FFC(-aptamer) denotes the control in which the FFC aptamer was replaced with a random-sequence aptamer. Error bars represent SD (n = 3)

AGG AAT GGC AAG CCA GGT AAG TTC CCA GAG CTC AAG CCA GGT TTT TGG GGG AAA AA-3'), for which no specific binding to FFC is expected. Under otherwise identical conditions, exposure to FFC produced no discernible change in LC texture and %Br, whereas the specific FFC aptamer yielded the characteristic %Br decrease. This result supports that the signal originates from FFC-aptamer complexation, which weakens interfacial association and promotes dehybridization/release at the aqueous/LC interface.

Real sample analysis

The applicability of the LC-based aptasensor was examined in food matrices relevant to residue screening. Raw milk and whole egg homogenates were extracted using a QuEChERS-type protocol (Food sample preparation), verified as FFC-negative by HPLC, and diluted 10× with PBS to mitigate viscosity and matrix-induced fouling. Matrix-matched spike recovery tests were prepared post-extraction at FFC concentrations of 0 (blank), 5, 10, 20, and 40 ng mL⁻¹ (final concentrations in the diluted extracts). Each level was analyzed in triplicate using the LC aptasensor and independently using HPLC under routine laboratory conditions.

Across both matrices, the LC aptasensor yielded recoveries of 97.80–105.15% with low repeatability errors (RSD ≤ 5%; Table 2). To statistically evaluate agreement between the LC aptasensor and HPLC, the concentrations obtained for the same spiked samples were compared using a two-tailed paired t-test. No significant difference was

Table 2 Spike recovery results of florfenicol in diluted food matrices

Sample	Amount added (ng mL ⁻¹)	Amount detected (ng mL ⁻¹)		Recovery (%)	RSD (%)
		HPLC	LC-sensor		
Milk	0	Non-detectable	Non-detectable	—	—
	5	4.87	5.12	102.40	4.62
	10	9.81	9.93	99.30	2.06
	20	19.67	21.03	105.15	5.01
	40	39.58	40.39	100.98	3.55
Egg	0	Non-detectable	Non-detectable	—	—
	5	4.92	4.89	97.80	3.87
	10	9.94	10.26	102.60	4.28
	20	19.68	20.54	102.70	3.85
	40	39.78	39.68	99.20	4.12

RSD relative standard deviation, HPLC high-performance liquid chromatography, LC liquid crystal

observed between the two methods ($p > 0.05$), supporting the absence of systematic bias within the tested concentration range. Collectively, these results indicate that, after simple dilution, the receptor-functionalized LC interface maintained quantitative performance in complex extracts and provided measurements consistent with HPLC, highlighting the practicality of the proposed method for rapid FFC screening in milk and eggs.

Conclusion

The proposed label-free LC-based aptasensor converts specific binding events between FFC and its aptamer into an optical response at the aqueous/LC interface. UV-induced oxidation of 5CB to CBA, followed by covalent conjugation of a complementary oligonucleotide, enables a receptor phase that supports aptamer hybridization and FFC-triggered dehybridization. Under optimized conditions, the sensor exhibited a linear response over 0.2–80 ng mL⁻¹ with an LOD of 0.12 ng mL⁻¹. The platform showed favorable selectivity toward FFC over structurally related veterinary drugs and common matrix components, together with good repeatability under the tested conditions (inter-chip replication, $k = 3$). In spiked milk and egg extracts following a simple QuEChERS-based pretreatment and dilution, the assay achieved recoveries of 97.80–105.15% with RSD ≤ 5%, and the measured concentrations were consistent with HPLC (paired t-test, $p > 0.05$). Owing to its label-free, enzyme- and fluorophore-free configuration, direct POM readout, and straightforward sample preparation, this LC-based aptasensor is suitable for rapid prescreening of FFC in food safety monitoring. Furthermore, the platform can be extended to other veterinary drug residues by substituting the recognition aptamer.

Authors' contributions Duong Song Thai Duong : conceptualization, formal analysis, writing – original draft, writing-review & editing. Chang-Hyun Jang : resources, supervision, funding acquisition, writing-review & editing.

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Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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