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# A liquid crystal biosensor for specific detection of antigens

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ABSTRACT

Following the principle of the enzyme-linked immunosorbent assay (ELISA) pathogen detection method, we demonstrate specific sensing of goat Immunoglobulin G (IgG) by a nematic liquid crystal material. Sensing occurs via the visually-striking realignment of a pre-fabricated liquid crystal film, suspended in grids and coated with biotinylated lipids followed by biotinylated anti-goat IgG. Realignment occurs when the targeted goat IgG is added to the cell, but not when rat or rabbit serum IgG is added to the same surface. In principle, this method can be generalized to provide an inexpensive, fast and sensitive prefabricated sensor for any pathogen.



## 1. Introduction

Currently, the most widespread technique to detect antigens is enzyme-linked immunosorbent assay (ELISA). This method uses a cascade of interactions between antibodies and antigens leading to a visual response that indicates the presence or absence of a given antigen or substance [1]. There are several different ELISA methods, such as direct and sandwich assays [2–4], but the underlying feature is the requirement for antibody–antigen binding to a surface followed by a secondary reaction to produce a response. This multi-step process always starts by binding the target antigen, either directly or via a capture antibody, to the surface of the assay chamber. The next step is blocking unbound sites with an unrelated protein-based solution. The third step is detection, where an antibody, conjugated with an enzyme such as horseradish peroxidase (HPR), binds the target antigen. The addition of a chromogenic substrate for the enzyme then creates a quantifiable signal. Washing processes separate each of these steps. Therefore, ELISA is a relatively lengthy process typically performed in a laboratory environment. Examples for other developing techniques for biosensors include surface plasmon resonance (SPR) [5] and microfluidics [6].

Liquid crystal materials have been used recently in a wide array of sensing applications that exploit the high sensitivity of their alignment to the conditions of surrounding immiscible media. Combined with the optical anisotropy of liquid crystals, this sensitivity produces a rapid, easily-visualized response. One of these techniques uses an aqueous non-toxic chromonic liquid crystal medium that reveals defects when an object in the medium is larger than the extrapolation length $b$, defined as the ratio of the director curvature elastic constant $K$ and the anchoring strength $W$ between the object and the liquid crystal medium [7,8]. If the size of the microbe before binding is smaller than $b$, but becomes larger after binding, a defect appears that can be detected optically. This technique, currently being developed by Crystal Diagnostics, Ltd. [9], provides rapid and selective identification of microbes.

The other type of liquid crystal-based sensing technique, pioneered by Abbott [10–14], utilizes realignment of the liquid crystal caused by absorption or desorption of surfactants at the LC-water interface. Pure water aligns the LC molecules to lie along the interface, whereas surfactants align them orthogonal to it. Although this technique has been demonstrated to be sensitive to a wide array of surfactants, lipids, DNA and proteins [15–19], it is very challenging to achieve the specific sensing of pathogens. In one variation of the techniques used by the Abbott group, solid surfaces are first functionalized with a layer that binds specifically to an antigen, and then any binding is detected by using the surface as an alignment layer for liquid crystal films. For example, the surface might be coated with biotinylated bovine serum albumin (BSA) that blocks non-specific adsorption of biological molecules but can bind to anti-biotin antigens, such as immunoglobulin G (IgG) [20]. A recent variant of specific detection used an antibody- or BSA-decorated LC aqueous interface. The LC alignment was shown to vary with specific interaction between vesicles and the antibody adsorbed at the LC interfaces [18].

None of these specific sensing methods offer a prefabricated sensor that can simply be brought into contact with an aqueous medium containing the antigen to be detected. A new pathogen detector in which

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the functionalized test substrates are made prior to the addition of the target antigen and the visualization can be done in remote locations, without the need of a laboratory environment, would be a significant advance. In this paper, we describe such a prefabricated sensor that holds the promise of providing inexpensive biosensors capable of visual confirmation of the presence of an antigen remote from any laboratory. We test this sensor design with several biomolecules, including controls.

## 2. Materials and methods

The liquid crystal used in our experiments, 4-cyano-4′-pentylbiphenyl (5CB, >99%), was obtained from Sigma Aldrich and used without further purification. However, any other nematic liquid crystal at convenient temperature ranges should be similarly useful. The lipids used were 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC; Avanti Polar Lipids, >99%) and a triethylammonium salt of N-((6-(biotinoyl)amino)hexanoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Biotin-X-DHPE; Life Technologies); both were supplied in the form of powders and used without further purification.

The mixed Biotin-X-DHPE and DLPC vesicles were prepared according to Patil and Jadhav [21]. The lipid powders were dissolved in reagent-grade chloroform (>99%, Sigma Aldrich) to prepare lipid stocks of ~25 mg/ml. Lipid films were prepared by mixing appropriate amounts of the stock solution and allowing the chloroform to evaporate off under vacuum at 30 °C. After adding 5 ml of ultrapure deionized water, at a temperature slightly above the gel-liquid crystal transition temperature, the liposome dispersion typically appears cloudy, indicating the presence of MLVs. The dispersion was either tip-sonicated for 10 min using the pulsing mode or extruded through a 200 nm poly(tetrafluoroethylene) filter to leave a clear solution, indicated that vesicles are much smaller than visible light wavelengths. The observed changes in the liquid crystal films were the same with both treatments, both in pattern and in dynamics. Fig. 1 presents the molecular structures of the liquid crystal 5CB and the lipids.

Pierce™ NeutrAvidin (deglycosylated avidin) was obtained from Life Technologies and kept suspended in Tris buffer at a concentration of 2 mg/ml. Phosphate buffered saline (pH = 7.4) was prepared in ultrapure deionized water (resistivity of 18.2 MΩ·cm) obtained through a PureLab Plus™ system. The antibody attached to the interface was biotinylated anti-goat IgG produced from either mouse or donkey serum (Sigma Aldrich, salt-free lyophilized powder in bovine serum albumin), and this was used as a sensor for non-biotinylated IgGs obtained from goat, rat, and rabbit sera (Sigma Aldrich). All materials were used without further purification.

The experimental setup was recently described in detail [14]. Fig. 2(a) shows schematics of the setup. The liquid crystal sensor is placed between either crossed linear or left- and right-handed circular polarizers and imaged with a CCD camera mounted on an inverted microscope. The liquid crystal is smeared uniformly in one half of a Veco folding nickel 50/100 mesh 20-μm thick TEM grids with cell diameters

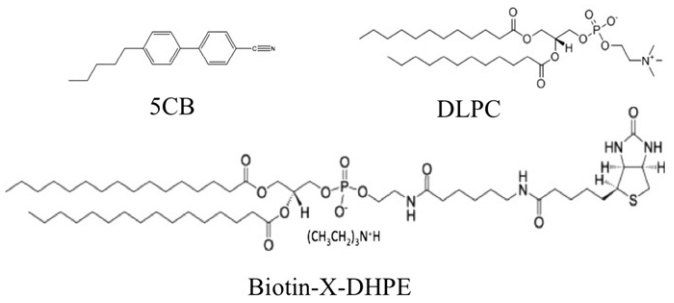


**Fig. 1.** Structures of 4-cyano-4′-pentylbiphenyl (5CB); 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC); N-((6-(biotinoyl)amino)hexanoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (Biotin-X-DHPE).

in the range of 0.2–0.4 mm. The liquid crystal forms stable freestanding films in each grid cell. Tweezers hold this grid parallel to the bottom of the dish for the aqueous material. When both sides of the film are in contact with air, the average orientation of the liquid crystal molecules ("director") aligns perpendicular to the interfaces ("homeotropic" alignment), which appears dark between crossed linear or left- and right-handed circular polarizers. The dish is then filled with water to make contact with the bottom of the grid. Pure water promotes a director alignment parallel to the interface ("planar" alignment). Since the alignment is homeotropic on the air side, hybrid alignment results. Biologically relevant materials are then added to the water, which may change the alignment and the optical properties of the liquid crystal film, providing the basis of sensing. A close-up view of the liquid crystal between air and aqueous interfaces is shown schematically in Fig. 2(b). Since the aqueous solution is optically isotropic, the picture observed in the inverted polarizing microscope depends only on the alignment of the liquid crystal. With hybrid alignment, the texture appears uniformly bright and colored between circular polarizers and usually inhomogeneous between linear crossed polarizers because that configuration is sensitive to the lateral distribution of the optical axis [14].

Images were collected using a QI Fast1394 camera attached to a modified Olympus CK-40 polarizing optical microscope. This microscope is equipped with interchangeable linear and circular polarizers and a microstage to hold the sample in place and allow for minute adjustments of the position of the sample. The camera was, in turn, connected to a computer equipped with QI Capture software to collect and save images.

## 3. Results

When amphiphilic phospholipids, such as 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) are added to water, they migrate to the LC/water interface with tails embedded in the LC orthogonal to the interface while exposing their headgroups to the aqueous medium. At sufficiently high (>1 mM) concentrations of phospholipid in the medium, this process rapidly leads to homeotropic LC alignment both on the air and liquid side. Once the lipid is adsorbed to the surface, the TEM grid is removed from contact with the dispersion and placed on top of a drop of deionized water for five or more minutes to rinse away any unadsorbed lipid. DLPC monolayers covering the LC/water interface remain stable, as indicated by a dark texture showing homeotropic alignment at both the air and water interfaces, even after rinsing the film with pure water, indicating that the DLPC molecules bound to the liquid crystal interface do not desorb into pure water. However, when the DLPC-decorated LC surface is put in contact with the protein apoLp-III which binds to phospholipids [22], the texture brightens, thus indicating a change of the alignment from the aqueous side [23]. This reveals that a binding process of the apolipoprotein with DLPC can be visualized by liquid crystals and encouraged us to test the response of an antibody-decorated LC surface to its antigen.

To construct a decorated liquid crystal interface to respond to specific antigens, we employed biotin–avidin binding, an extremely strong interaction used in labeling of antibodies [24]. Fig. 2(c) illustrates the cascade of biotinylated lipid, avidin, biotinylated anti-goat (targeting) IgG and goat (targeted) IgG adsorbed at the liquid crystal–water interface described below. At each step of fabricating the sensor and testing it against different antibodies, the liquid-crystal filled grid was rinsed with ultrapure deionized water, and then gently lowered onto a 0.5 ml droplet of the desired dispersion.

First, we prepared vesicles containing 2 wt.% Biotin-X-DHPE in DLPC and added water to create a vesicle suspension with a 1 mM lipid concentration. Unlike in other experiments where sensing the presence of the lipid is the primary aim [11,14,19], the goal here was merely to produce a dense lipid layer adsorbed to the surface. Fig. 3(a–c) shows the typical progression of film appearance upon addition of the lipid vesicles. Before the lipid reaches the LC interface, the texture is uniformly