Liquid-Crystal Biosensors

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Signal-Enhanced Liquid-Crystal DNA Biosensors Based on Enzymatic Metal Deposition**

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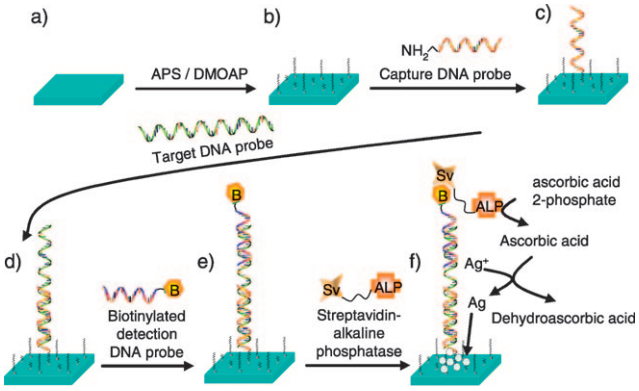
Liquid crystals (LCs) are materials that can exhibit the mobility of liquids and the anisotropy of solid crystals. The long-range orientational order and optical anisotropy of LC molecules can transform chemical and biomolecular binding events into amplified optical signals that can be easily observed, even with naked eye.[1–5] Since Abbott and co-workers[1] initiated the field of study using LCs as sensing elements in the detection of biomolecules, liquid-crystal-based biosensing detection has attracted particular attention, because it can localize biomolecules to specific regions of a substrate with micrometer resolution, and the procedure can be carried out under ambient lighting even without the need for electrical power. Specifically, it is of great potential for providing highly sensitive and low-cost bioassays performed away from central laboratories.[6–18]

Abbott and co-workers[1,2,6,7] reported that parallel-rubbed bovine serum albumin film and obliquely deposited nanostructured gold film can aid the alignment of LCs to the direction of rubbing or depositing, the specific biomolecular binding events (such as antibody–antigen, protein–ligand, or protein–protein recognition events) can mask the nanostructured grooves, leading to distinguishable orientations of LCs supported on these surfaces. Yang and co-workers[8] developed protein assays on plain glass substrates coated with an organosilane for inducing the homeotropic alignment of LCs. The method can conveniently provide a yes/no answer when the protein concentration exceeded a critical value. Notwithstanding the versatility and simplicity of these LC biosensing approaches based on biomolecular binding events, the sensitivity is limited by the size and amount of biomolecules. This factor may restrict the LC biosensing technique in the bioassay of low concentrations or trace analytes. Ultrasensitive detection of specific DNA sequences is a field of ever-increasing interest[19–24] for clinical diagnostics, gene therapy, and a variety of other biomedical applications. Due to the $\mu g\,mL^{-1}$ or nM detection limits,[9–12] however, the LC biosensing technique based on direct biomolecular binding events is difficult to meet the demand of ultrasensitive DNA assays. There is thus significant interest in seeking signal-enhancement strategies for the LC-based DNA biosensing techniques to circumvent the problem of detection sensitivity.

Herein, we exploit a highly sensitive signal-enhanced LC biosensing technique based on enzymatic metal silver deposition. A specific DNA sequence assay was chosen as a proof-of-concept model (Scheme 1). First, a chemically functionalized surface on a plane glass slide is obtained by self-assembling a (3-aminopropyl)trimethoxysilane (APS)/*N*,*N*-dimethyl-*N*-octadecyl(3-aminopropyl)trimethoxysilyl chloride (DMOAP) film. DNA immobilization was then achieved by binding a capture DNA probe to the APS/DMOAP film by a cross-linker, followed by hybridizations of a target DNA and a biotinylated detection DNA probe. Subsequently, the streptavidin alkaline phosphatase (Sv-ALP) was bound to the biotin of the detection probe, which then catalyzes the hydrolysis of ascorbic acid 2-phosphate (AA-p) to form ascorbic acid. The latter, in turn, reduces the silver ions in solution to form the deposition of metallic silver on the substrate surface.[25,26] In comparison with the existing LC biosensing methods established on the disruption of LC orientation by direct biomolecular binding events, the sensitivity of the proposed method depends on the condition of the enzymatic reaction instead of the size and amount of the biomolecules. Moreover, the background signal of the metallization is minimized, as the reducing agent is formed by enzymatic reaction rather than initially provided in the solution. These advantages allow higher sensitivity for DNA



Scheme 1. Stepwise assembly of the signal-enhanced LC DNA biosensing substrate based on enzymatic silver deposition. a) Cleaned glass slide; b) self-assembled APS/DMOAP film; c) immobilization of capture DNA probe; d) hybridization with target DNA; e) hybridization with biotinylated detection DNA probe; f) association with streptavidin alkaline phosphatase and reduction of silver ions by ascorbic acid.

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detection. Therefore, the silver enhancing method would offer highly sensitive detection for varieties of analytes and play a crucial role in expanding the application scope of the LC biosensing technique.

The surface modification of LC cells plays a key role in the orientation of LCs and has a great influence on the signal-to-noise ratio and the distinction between positive and negative results. Previous studies have reported that the mixed monolayers formed by both long and short alkanethiols can cause perpendicular alignment of 5CB.[2,27] Herein, the LC cells were fabricated using both APS/DMOAP-decorated surfaces and DMOAP-coated glass slides, which not only have surface amino groups for coupling of DNA probes or proteins, but can also orientate LCs homeotropically to yield a dark background.[27–30] The optical images from a polarized-light microscope showed that the number of bright spots in the optical images decreased with the decrease of APS/DMOAP ratio, and a uniform dark background appeared when the APS/DMOAP ratio was reduced to 5:1 (Figure 1).

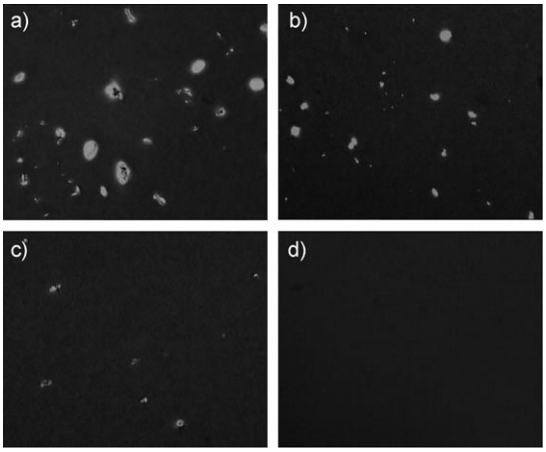

Figure 1. Optical images under crossed polarizers of LC cells with 5CB sandwiched between a DMOAP-coated glass slide and an APS/DMOAP-decorated glass slide. The APS-DMOAP ratios were a) 50:1, b) 20:1, c) 10:1, and d) 5:1.

An atomic-force microscope (AFM) image showed that the APS/DMOAP self-assembled film had a uniform surface morphology, and the largest peak-to-valley-height (LPVH) was 6.31 nm (see the Supporting Information). The thickness of the film showed that double layers of APS and DMOAP formed on the surface compared with the theoretical thickness of the DMOAP monolayer (3.0 nm, molecules standing upright).[31] The water contact angle measurements further revealed that the water contact angle of the surfaces increased with the decrease of the APS-DMOAP ratio, reached 85° at the APS/DMOAP ratio of 5:1 (sufficient to induce homeotropic alignment of LCs), and then tended to be stable at lower ratios. The lower ratios of APS/DMOAP had little effect on the inducing activity of surface-to-LC perpendicular alignment, but would reduce the coverage of amine groups, which is not beneficial for the biomolecular immobilization.

Recent research has revealed that although a high concentration of protein can greatly disrupt the uniform optical appearance of LCs, the presence of low protein concentrations causes only slight disruption.[6–8,32] To investigate the effect of enzymatic silver deposition on the alignment of LCs, Sv-ALP was bound to the APS/DMOAP film surface by a cross-linker glutaradehyde by covalent bond formation, and then metallic silver was deposited on the glass slide surface by addition of substrate solution. An optical image of the LC cell made from a glass slide modified by Sv-ALP with concentration as high as 0.1 $\mu g\,mL^{-1}$ (Figure 2) was

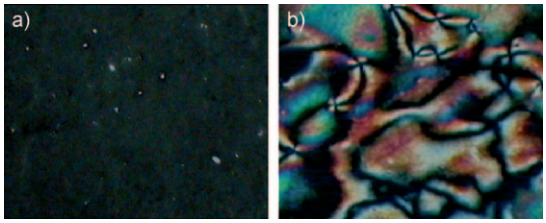

Figure 2. Optical images under crossed polarizers of LC cells with 5CB sandwiched between a DMOAP-coated glass slide and a Sv-ALP-modified glass slide. The optical images were obtained a) before and b) after silver deposition. The concentration of Sv-ALP was 0.1 $\mu g\,mL^{-1}$; the substrate solution contained 1.5 mM AA-p and 1.0 mM $AgNO_3$. The silver deposition was performed at 37 °C for 30 min.

still uniformly dark except few small bright spots, suggesting that a homeotropic orientation of LCs at low concentration of Sv-ALP. In contrast, the optical image of the LC cell made from a silver-deposited glass slide displayed obvious birefringent domains. This result demonstrated the effectiveness of deposited silver for optical amplification.

Previous theoretical and experimental studies have demonstrated that the orientation of LCs close to a substrate surface are balanced by the chemical composition and topographical structure of that surface.[4,33] Changes in either the chemical composition of the surface or its topographical structure may break the balance and thus result in a corresponding change in the LC orientation.[28] To further understand what caused the apparent differences between Figure 2a and Figure 2b, scanning-electron microscopy (SEM) was applied to characterize the exterior state of the substrates before and after silver deposition. As shown in Figure 3a,b, there was no appreciable distinction in the exterior structure of APS/DMOAP-coated substrates modified with or without Sv-ALP. This result indicated that the very low surface density of Sv-ALP had little effect on the topographical structure of substrate surface, and the long

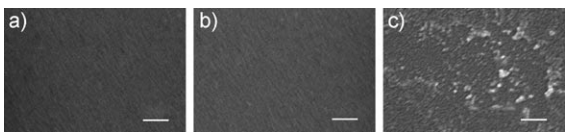

Figure 3. SEM images of Sv-ALP-modified glass slide a,b) before and c) after silver deposition. The concentrations of Sv-ALP were a) 0, b,c) 0.1 $\mu g\,mL^{-1}$. The enzyme reaction buffer contained 1.5 mM AA-p and 1.0 mM $AgNO_3$. The silver deposition was performed at 37 °C for 30 min. Scale bars: 100 nm.

alkyl chain layer of DMOAP still predominated in inducing the homeotropic alignment of LC molecules (Figure 4a,b). In contrast, a large number of silver nanoparticles were assembled on the substrate by enzymatic reaction (Figure 3c),

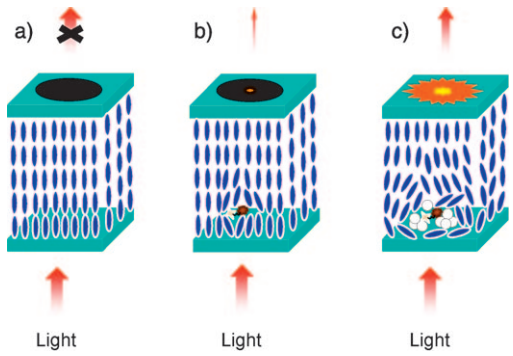

Figure 4. The orientations of 5CB in the LC cells fabricated with the DMOAP-coated glass slides (upper slides) and modified slides (a–c; lower): a) APS/DMOAP-coated glass slide, b) Sv-ALP-modified glass slide, and c) silver-deposited glass slide.

which greatly changed the surface topology and thus induced a homeotropic-to-tiled transition of the LC molecules surrounding them (Figure 4c). Due to the long-range order inherent in LC phases, the homeotropic-to-tiled transition of the LC molecules would then cause a distorted orientational profile inside the LC cell, making the optical image of LC cell birefringent as a result.

To study the optical responses of LCs triggered by oligonucleotides, the target DNA probe was hybridized with the capture DNA probe immobilized on the APS/DMOAP film surface by a cross-linker glutaradehyde. The results showed that the optical appearances of LC cells had some bright spots in the dark background until the concentration of target DNA increased to 1 nM (images not shown). It can be considered that a low surface density of oligonucleotides can only slightly disrupt the orientations of LCs and produce a weak response in the optical textures.

Now it has been shown that the direct DNA hybridization assay cannot meet the need of highly sensitive detection, the enzymatic silver deposition as a signal enhancement strategy was applied to decreasing the detection limit for target DNA (Scheme 1). The amount of the coupled Sv-ALP increased with the increase of the target DNA concentration (Figure 5), and thus more silver nanoparticles were catalytically deposited on the substrate, resulting in more birefringent textures in the optical appearances of LC biosensors. Obviously, the deposition of silver nanoparticles caused a significant decrease in the detection limit from 1 nM to 0.1 pM. The response was saturated when the concentration of the target DNA reached 100 pM. It should be noted that the proposed method lowers the detection limits by four orders of magnitude and has a higher DNA detection sensitivity compared with previous reports.[9–12]

The selectivity of the proposed DNA biosensor was investigated by using enzymatic silver deposition to amplify the signals of the completely complementary target DNA sequences and the two-base mismatched DNA sequences with the same concentration. As shown in Figure 5f, the optical signal for two-base mismatched sequences was significantly weaker than that of the complementary sequences, demonstrating its good selectivity.

In conclusion, we have proposed a novel signal-enhanced liquid-crystal biosensing approach based on enzymatic silver deposition for the highly sensitive detection of DNA. The mixed self-assembled film of APS/DMOAP was demonstrated to be an effective biosensing substrate for LC biosensors, which provides functional amino groups for biomolecular immobilization by covalent bond formation and also long alkyl chains for LC homeotropic alignment by short-range interactions. The enzymatic silver nanoparticles deposited on the LC sensing substrate greatly changed the surface topology and further induced a homeotropic-to-tiled transition of the LC molecules surrounding them, resulting in an obvious change of the optical appearances of LC biosensors from a dark background to a birefringent texture before and after enzymatic silver deposition. This effect revealed that the homeotropic-to-tiled transition of LCs are caused by the deposited silver, an excellent signal enhancement element. The combination of enzymatic signal amplification and LC-based imaging contributed a highly selective and ultrasensitive method for the detection of DNA (the detection limit is about 0.1 pM).

Although there have been some reports on the amplification detection of DNA hybridization in other fields,[19–24,34–39] to our knowledge, this is the first demonstration of enzymatic signal enhancement in the field of LC biosensors. The proposed signal-enhanced LC biosensing

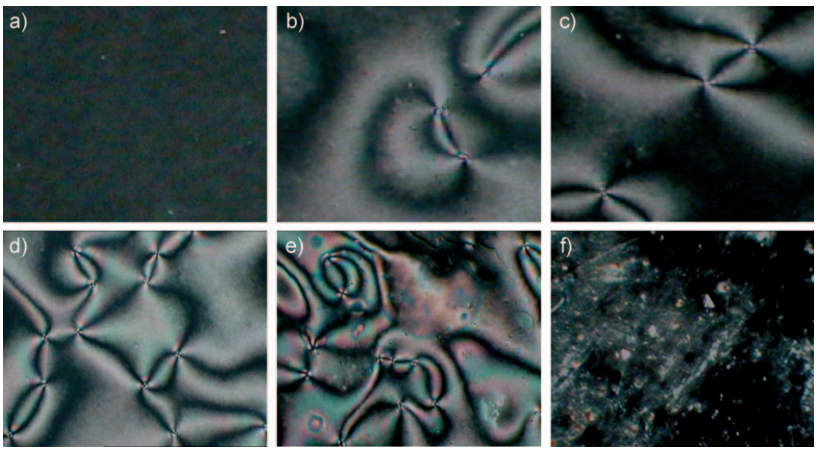

Figure 5. Optical images under crossed polarizers of LC cells with 5CB sandwiched between a DMOAP-coated glass slide and a silver-deposited glass slide with complementary target DNA at concentrations of a) 0, b) 0.1, c) 1, d) 10, e) 100 pM, and f) two-base mismatched DNA at concentration of 100 pM.

approach is also a promising tool for the study of some specific biomolecular interactions by various types of binding events (for example, antibody–antigen, hormone–receptor, and protein–receptor interactions) and will play a crucial role in expanding the application scope of the LC biosensing technique.

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