Optical detection of deoxyribonucleic acid hybridization using an anchoring transition of liquid crystal alignment

Hak-Rin Kim and Jae-Hoon Kim
Department of Electronics and Computer Engineering, Hanyang University, Seoul 133-791, Korea
Tae-Sun Kim
Department of Chemistry, Hallym University, Chuncheon, Kangwon-Do 200-702, Korea
Sang-Wook Oh and Eui-Yul Choi
Central Research Institute of Boditech Inc. and Department of Genetic Engineering, Hallym University, Chuncheon, Kangwon-Do 200-702, Korea

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We demonstrate an optical method for detecting specific binding events in an oligo deoxyribonucleic acid (DNA) chip using an anchoring transition of a nematic liquid crystal (NLC) as a result of DNA hybridization. A homeotropic orientation of the NLC supported by oligoDNA changes to a random planar orientation after hybridization. Such DNA hybridization and subsequent NLC reorientation are easily observable with a simple detection system via long-range orientational order and large optical anisotropy of the NLC. © 2005 American Institute of Physics.
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Recently, several types of biosensors have been demonstrated for analyzing and obtaining various genetic information using simple techniques. To detect biological events, biosensors generally combine a bioreceptor and a transducer. The bioreceptor recognizes the specific biomolecular interactions via various types of binding events, such as the deoxyribonucleic acid (DNA) hybridization process, antibody-antigen interactions, hormone-receptor interactions, and protein-receptor interactions.1–4 The transducer converts the recognition of the binding into a quantitative signal. As a transducer, most of biosensors adopt a fluorescent or radioactive probing technique.5 However, such approaches require expensive and sophisticated analysis procedures with laboratory-based equipment. Thus, it has taken considerable efforts to develop an efficient and simple detection method.6

Recent research7–10 has shown that the delicate interplay between liquid crystals (LCs) and modified surface can be used for visual signal detection in a ligand-receptor binding or chemical exposure system. It is known that a properly treated surface can preferentially orient the LC molecules in a certain direction as a result of anisotropic interfacial interactions between the LC molecules and the surface.11 Moreover, the long-range orientational order and large optical anisotropy of the LC can transform various surface events into amplified optical signals that can be easily observed without expensive detection systems.7,9,10

In this letter, we propose a novel method for detecting hybridization results in a DNA chip using an anchoring transition of LC alignment. To investigate the effects of structural changes of DNA on the LC alignment, we used a functional substrate on which single-stranded oligonucleotide DNA (ssDNA) was immobilized to the surface. It was found that initial homeotropic LC orientation on the ssDNA interface was changed to a planar inhomogeneous LC orientation on the double strand DNA (dsDNA) interface after hybridization. Such hybridization events were effectively transformed into optical signals via the LC layer, which could be observed by naked human eye with only a simple crossed polarizer system.

Our functional substrate was made by selectively immobilizing oligoDNA onto a Biotin Chip™ glass substrate. To prepare the functional substrate, we immobilized the capture oligoDNA onto the Biotin Chip™ pretreated with a Calix-arene derivative, which was developed as a molecular linker to a biotin (Biometrix Technology, Korea). Thus, any biotin-conjugated oligoDNAs can be easily assembled on the modified solid surface of the glass slides, the Biotin Chip™. The linker molecule interacts with biotin by molecular recognition via hydrogen bonds. A 19-mer oligoDNA of p53 tumor suppressor gene containing an extra biotin (5'-biotin-gctgaggtctggtttgca-3') was used as a model system in this study. To immobilize the oligoDNA, 1 μL of oligoDNA (1.5 nmol/mL) in blocking solution (4X SSC, 15% glycerol; 1X SSC, 8.8 g of NaCl, and 4.4 g of sodium citrate in 1000 mL H2O) was manually spotted onto the Biotin Chip™ glass substrate with a micropipet by a diameter of 0.5 cm and incubated for 2 h at room temperature. After immobilization, the glass slide was washed with 2X SSC, 0.1% SDS solution for 1 min, followed by 0.1% SSC solution for 1 min, and then dried under a stream of N2 gas. The orientation of the immobilized oligoDNA was characterized by measuring the retardation variation under the sample rotation using a He–Ne laser and a photoelastic modulator (PEM 90, Hinds Instruments). The measured optical retardation of the immobilized oligoDNA was symmetrically increased as an inclination angle of the substrate with respect to the plane of incidence increased. This means that the immobilized oligoDNAs were aligned in a direction normal to the surface of the Biotin Chip™ substrate.

The hybridization effect of the immobilized oligoDNA on the anchoring transition of the LCs was characterized by preparing two types of LC cells as shown in Fig. 1. One LC cell was assembled with an unmodified glass substrate and the Biotin Chip™ glass substrate, which had selectively deformed by the immobilized oligoDNA. The other LC
cell was assembled after the immobilized oligoDNA was hybridized by its complementary partner oligoDNA (5'-ttgcaacggtccttagacg-3'). To show the molecular recognition between the capture and the target DNA, complementary oligoDNA (0.5 nmol/mL in solution (4X SSC, 0.1% SDS, 15% glycerol) was overloaded on the spot that the biotin-oligoDNA (1.5 nmol/mL) had previously been immobilized, and then the glass slide was incubated for 30 min at 50 °C for hybridization reaction. After hybridization, the glass slide was washed with the same surface treatment as previously described. Each LC cell was 2.5 cm × 1.5 cm. A nematic LC (NLC) material, 5CB (4'-pentyl-4-cyanobiphenyl) of E. Merck, was then filled into the sandwiched sample cell at isotropic temperature. The phase transition sequence of 5CB used in our experiment is crystal → (24 °C) → nematic → (35.3 °C) → isotropic phase. The cell gap was maintained using glass spacers of 10 μm. As shown in Fig. 1, to observe birefringent NLC orientation before and after hybridization of the immobilized oligoDNA, only a set of crossed polarizers was required.

Figure 2(a) is a schematic view of the microscopic observation in our oligoDNA chip. In Fig. 2, the surface of Region I has been modified by the homeotropically immobilized oligoDNA or its duplex DNA, whereas Region II has the only biotin layer and no surface modification. In Region II of Figs. 2(b) and 2(c), the microscopic texture of the sample appeared white, which meant that the LCs were aligned in a planar inhomogeneous orientation to the glass substrate (i.e., Schlieren texture). On the surface with immobilized oligoDNA (Region I), the NLC alignment texture was homeotropically orientated, thus the image appeared black under the crossed polarizers as shown in Fig. 2(b). However, after hybridization of the oligoDNA with its complementary partner, the texture was in the white state again [Fig. 2(c)]. This result means that a selective biomolecular recognition event was transferred to the LC anchoring transition event via an intermolecular interaction between the biomolecular surface and the surface LC molecules.

FIG. 1. The oligoDNA chip structure for optically detecting DNA hybridization by anchoring transition of a NLC layer. A Biotin Chip™ substrate is selectively immobilized with ssDNA or dsDNA in Area I.

![Image](image.png)

FIG. 2. Microscopic images of the sample: (a) The scheme for the observation system; (b) and (c) the microscopic LC alignment textures of the samples before and after the p53 oligoDNA immobilized in Area I is hybridized by its complementary partner oligoDNA, respectively.

![Image](image.png)

Figure 3 illustrates the anchoring transition that depends on the packing density of the surface biomolecular layer. Before hybridization, the immobilized oligoDNA acts as a homeotropic alignment layer through a similar anchoring effect of an amphiphilic monolayer such as lecithin. When the molecular lengths of our 19-mer oligoDNA and NLC molecules are assumed to be approximately 68 Å and 15–20 Å, respectively, a sufficient steric interaction between the NLC molecules and the homeotropically immobilized oligoDNA is expected in our system. However, at higher packing density after the hybridization process, the amount of infiltrated NLC molecules decreases due to the reduced free space between the immobilized duplex DNAs. This results in our homeotropic-to-planar inhomogeneous anchoring transition. It should be noted that our results of anchoring transition in the LC layer, depending on the binding events of oligoDNAs, take place in the NLC molecules which do not have a smectic A phase in the phase transition sequence due to a screening effect of the highly energetic surface smectic layer.

Our immobilized oligoDNA substrate works for a multifunctional interface. One function is as a bioreceptor using a specific binding event depending on the structure of the immobilized oligoDNA. The other function is as a transducer that converts the specific hybridization events into amplified optical signals via the LC aligning effect. Although the hybridization events of the DNA and the subsequent molecular interaction between the immobilized DNA and the NLC molecules take place within a layer whose thickness is in the tens of nm, such binding events can be communicated to the NLC bulk beyond a distance of tens of μm through the long-range elastic deformation of the NLC molecules. Thus, the hybridization events of the DNA and the subsequent molecular interaction between the immobilized DNA and the NLC molecules take place within a layer whose thickness is in the tens of nm, such binding events can be communicated to the NLC bulk beyond a distance of tens of μm through the long-range elastic deformation of the NLC molecules.
bridization results can be read by the naked eye as shown in the real sample images in Fig. 4. Figures 4(a) and 4(b) were obtained before and after the hybridization event, respectively.

We prepared a NLC-based DNA chip array in which the oligoDNA was selectively spotted in a diameter size of 50 μm by a microarrayer in 2×2 matrix. Then, the oligoDNAs on the second row of the 2×2 array were hybridized.

Figure 5(a) depicts the microscopic NLC alignment texture, which shows homeotropic and homogeneous alignment on the first and the second row of the immobilized array, respectively. Figures 5(b) and 5(c) give the relative optical intensity measured at the cross sections along the first row and the second column of the 2×2 matrix oligoDNA chip, respectively. The extinction ratio of the first row to the second row was approximately four, which was enough to distinguish the hybridized results with a simple optic system.

In summary, we have proposed a simple optical detection system for specific hybridization results of the selectively immobilized oligoDNA array. In our experiment, we used a 19-mer oligoDNA of p53 tumor suppressor as a bioreceptor and its complementary partner oligoDNA as a target material. Using the anchoring transition of the NLC, which depends on whether the DNA structure on our functional surface was single or double stranded, it was demonstrated that specific DNA binding events could be easily observed with less expensive equipment than is required by conventional detection schemes. The LC anchoring properties on the homeotropically immobilized DNA were very similar to that on the conventional amphiphilic homeotropic surfactant. Thus, it is expected that a quantitative analysis of dehybridization as well as hybridization process of DNA can also be explored.

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