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Gold nanoparticle based signal enhancement liquid crystal biosensors for DNA hybridization assays†

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A novel signal enhanced liquid crystal biosensor based on using AuNPs for highly sensitive DNA detection has been developed. This biosensor not only significantly decreases the detection limit, but also offers a simple detection process and shows a good selectivity to distinguish perfectly matched target DNA from two-base mismatched DNA.

The sensitive and selective detection of nucleic acids is important in biological studies, clinical diagnostics, and genetic therapy.[1,2] The development of DNA biosensors has increased tremendously over the past few years. Several types of biosensors have been demonstrated for analyzing various genetic information, including optical, electrochemical, and gravimetric DNA biosensors.

Recently, liquid crystal (LC) biosensors have attracted particular attention for their unique properties. Liquid crystals are remarkable materials which have a high degree of shape, dielectric, and optical anisotropy.[3–14] The alignment of LC molecules is extraordinarily sensitive to biomolecular and chemical bounding events, and the long-range order inherent in LC phases serves to amplify surface-disrupted ordering for macroscopic distances.[3,8] These idiosyncrasies, combined with the optical anisotropy of LC molecules, make them well-suited as 'sensing elements'. As early as 1998, Abbott and co-workers initiated a field of study concerned with using LCs as sensing elements in the detection of biomolecules.[3] In spite of the versatility and simplicity of these LC biosensors based on direct biomolecular binding events, the relatively low sensitivity at micromole or microgram levels of DNA is a detriment and limits their application in bioassays of low concentration or trace analytes. Since the DNA sequences of interest may be present in very small amounts, it is necessary to develop amplification techniques that enable the detection of trace levels of specific sequence.

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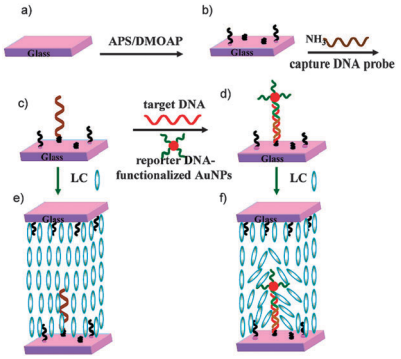
The development of signal amplified approaches is an efficient way to improve the sensitivity of LC biosensors. Recently, our group has reported a signal enhanced LC biosensing approach based on enzymatic silver deposition for highly sensitive DNA detection. The enzymatic silver nanoparticles have a far bigger geometrical size than these binding biomolecules and can greatly change the surface topology and further result in an enhanced optical signal.[15] The method has lowered the detection limits to 0.1 pM. Although the enzymatic silver deposition is demonstrated to be effective to enhance the optical signal of LC biosensing, the additional enzymatic process makes the LC biosensing procedure relatively complicated. Since gold nanoparticles (AuNPs) have been successfully used to improve the sensitivity of DNA bioassays for their facile synthesis, large specific surface area, high chemical stability, favorable biocompatibility, and high affinity of binding to amine/thiol-containing molecules,[16] we think that they should be effective in the enhancement of LC biosensing signals by the direct introduction of AuNPs with a relatively large specific surface area which can accommodate large numbers of DNA strands on their surface to increase the disruption to the LC orientation.

In this study, we propose a simple LC biosensing strategy for the highly sensitive detection of DNA by using AuNPs as the signal amplification element. This method sets detection and signal amplification at the same time. To demonstrate the signal amplification strategy, a specific DNA sequence assay was chosen as a proof-of-concept model (Scheme 1). Firstly, a chemically functionalized surface on a plain glass slide was obtained by self-assembling an (3-aminopropyl)trimethoxysilane (APS)/*N*,*N*-dimethyl-*N*-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP) film, which cannot only provide plentiful amino groups for coupling with DNA probes but also orientate LCs perpendicularly to provide a dark background. Then, the DNA immobilization was performed by binding the capture DNA probe to the APS/DMOAP film through a glutaraldehyde (GA) cross-linker, followed by hybridization of the target DNA and reporter DNA-functionalized AuNPs. Subsequently, the LC cells were fabricated using DMOAP-coated glass and APS/DMOAP-coated glass, and 5CB was drawn into the LC cell by capillary action. In comparison with the existing LC biosensing methods established on the disruption of LC orientation *via* direct binding events, since a single AuNP is loaded with hundreds of



Scheme 1 Stepwise assembly of the signal enhanced DNA LC biosensor based on AuNPs. (a) Cleaned glass slide, (b) self-assembled APS/DMOAP film, (c) immobilization of capture DNA probe, (d) hybridization with target DNA and with reporter DNA-functionalized AuNPs, (e) the orientations of 5CB in the LC cells fabricated with the DMOAP-coated glass slide (upper) and the APS/DMOAP-coated glass slide (lower) with capture DNA probe, (f) the orientations of 5CB in the LC cells hybridized with target DNA and with reporter DNA-functionalized AuNPs.

reporter DNA strands and the areal density of DNA is obviously increased, these will bring about a great change in the topographical features of LC biosensing surfaces and greatly increase the sensitivity of LC biosensors. This amplification LC biosensor can detect down to 0.1 pM of DNA, which brings about an amplification factor of 4 orders of magnitude compared with the prevenient DNA liquid crystal biosensing approach (1 nM). Moreover, the proposed LC biosensing method is quite simple since the whole detection process needs only one-step hybridization, and possesses the potential to detect varieties of DNA analytes.

A key step for the orientation of LCs is the surface modification of LC cells which has great influence on the background signal. Some studies have reported that the mixed monolayers formed by both long and short alkane thiols can cause vertical alignment of 5CB.[4,17] Herein, a mixed monolayer of APS and DMOAP was employed to build the DNA LC biosensing surface. Part of the glass slides was first immersed in a sodium acetate/acetic acid buffer (10 mM, pH 5.0) containing various ratios of APS and DMOAP to perform the self-assembly modification. The LC cells were then fabricated by using one mixed APS/DMOAP-coated glass slide and one DMOAP-coated glass slide. As can be seen from Fig. 1, the number of bright spots in the optical images decreased with the decrease of the APS/DMOAP ratio, and a uniform dark background appeared when the APS/DMOAP ratio was reduced to 5 : 1. It is known that the orientation of LCs close to a substrate surface is not only affected by the surface chemical composition and topographical structure but also affected by the surface energy.[18] Changes in either the chemical composition of the surface or its topographical structure may break the balance and therefore result in a corresponding change in the LC orientation.[19] An AFM image (see Fig. S3 in the ESI†) shows that the APS/DMOAP-coated glass slides have an approximately uniform surface morphology, and the maximum peak-to-valley-height (LPVH) is 6.31 nm. This peak–valley topographical appearance, which is relative to the mixed self-assembly of APS/DMOAP with long and short alkyl chains,

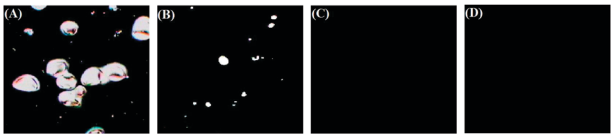


Fig. 1 Optical images (under crossed polarizers) of LC cells with 5CB sandwiched between a DMOAP-coated glass slide and an APS/DMOAP glass slide. The APS/DMOAP ratios were (A) 20 : 1, (B) 10 : 1, (C) 5 : 1, and (D) 1 : 1.

allows perpendicular embedding of LC molecules, further inducing the oriented perpendicular alignment of body LC molecules in the LC cell. Water contact angle measurements were also performed and revealed that the water contact angle of the surfaces increased with the decrease of the APS/DMOAP ratio (see Fig. S4 in the ESI†), reaching 80° at the APS/DMOAP ratio of 5 : 1. The lower ratios of APS/DMOAP had little promotion for the inducing activity of surface to LC perpendicular alignment, but would reduce the coverage of amine groups and not benefit the biomolecular immobilization. These values are in reasonable agreement with a previous study which found that the orientation of 5CB on a surface changed from homeotropic to tilted or planar alignment when the water contact angle was below 64°.[20] Therefore, the APS/DMOAP ratio of 5 : 1 was employed in the following experiments. The amino-labeled capture DNA probe was immobilized on the as-prepared APS/DMOAP-modified glass slides by cross-linkage with GA. The vertical barrier formed by the DMOAP makes the immobilized dialdehyde molecule adopt an orientation perpendicular to the surface and enables its second aldehyde group to react with an amino-labeled capture DNA probe.

Fig. 2 shows the optical textures of 5CB in contact with surfaces on which the capture DNA probe and the reporter DNA probe were hybridized to different concentrations of target DNA in a 'sandwich' format. The optical appearance of the LC cells under crossed polarizers shows that some bright spots and interference colors of the LC cells can observed in the regions where over 1 nM of target DNA was immobilized and that the birefringent domains increase with the concentration of target DNA. On the other hand, the low concentration of target DNA (0.1 nM) and the control experiment (with buffer only) are dark. It can be concluded that these bright spots indicate the near-planar anchoring behavior of LC molecules at the immobilized DNA probe laden interface of a LC cell.[21] The complex and non-uniform appearance of LCs reflects the intense disruption of the orientation profile in the thin LC layer. In the absence of immobilized DNA probes or at the lower concentration of DNA, the orientations of the LCs remain homeotropic such that the optical appearance of the LCs is still dark. It seems that the high density of DNA can obviously disrupt the orientation of LCs, but the low areal density of DNA can

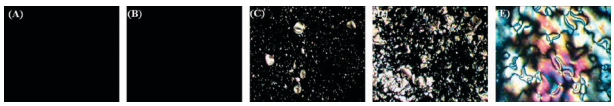


Fig. 2 Optical images (under crossed polarizers) of LC cells with 5CB sandwiched between a DMOAP-coated glass slide and an APS/DMOAP glass slide with immobilized DNA. The target DNA is at concentrations of (A) 0.0 nM, (B) 0.1 nM, (C) 1 nM, (D) 10 nM, and (E) 100 nM.

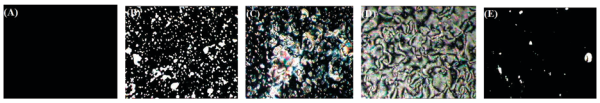


Fig. 3 Optical images (under crossed polarizers) of LC cells based on reporter DNA-functionalized AuNPs. Complementary target DNA at concentrations of (A) 0; (B) 0.1 pM; (C)1 pM; (D) 10 pM; and the two-base mismatched DNA at a concentration of (E) 10 pM.

hardly disrupt the orientation of the LCs and produce a negligible response in the optical textures. This result means that such a simple optical detection system based on biomolecular binding events can be easily used for the specific DNA assay, but the relatively low sensitivity hardly meets the requirements of modern biological analysis.

Since the orientations of the LCs are influenced by the density of DNA, the increase in the surface density of DNA should be an efficient way to enhance their disruption behavior to LC orientation and to improve the sensitivity of LC biosensors. Previous reports have demonstrated that AuNPs with diameters around 10 nm could accommodate 50–100 DNA strands on their surface,[22] and the high curvature of 12 nm AuNPs enabled the loading of single stranded DNA with a 100 times higher density than on a flat Au surface.[23,24] This means that the areal density of DNA owing to the specific DNA hybridization events will greatly increase when DNA functionalized AuNPs with numerous DNA strands are used as the reporter DNA strands, which is an advantage to enhance the LC biosensing signal.

To investigate the amplifying effect of the reporter DNA-functionalized AuNPs, the capture DNA probe was immobilized on the GA-activated APS/DMOAP mixed layers, and the different concentrations of target DNA and reporter DNA-functionalized AuNPs were hybridized to the capture DNA probe in turn. The optical appearance of the LC cells under crossed polarizers shows that some bright spots and interference color of the LC cells can observed in the regions even if 0.1 pM of target DNA was used, and the birefringent domains increased with the concentration of the target DNA (Fig. 3). This result indicates that the AuNP-based sensing strategy brings about an amplification factor of 4 orders of magnitude, resulting in a significant decrease of the detection limit from 1 nM to 0.1 pM. It should be noted that the proposed method has a significantly lower detection limit compared with that reported in previous work on direct DNA hybridization approaches.[11,15,25,26]

The selectivity of the proposed DNA biosensor was also investigated by using reporter DNA-functionalized AuNPs to amplify the signals of the completely complementary target DNA sequences and the two-base mismatched DNA sequences with the same concentration, respectively. As shown in Fig. 3E, the optical signal for two-base mismatched sequences was evidently weaker than that of the complementary sequences, demonstrating its good specificity.

In summary, we have proposed a novel signal enhanced LC biosensing approach based on AuNPs for highly sensitive DNA detection, in which as low as 0.1 pM target DNA can be detected. The AuNP-mediated DNA can greatly change the surface density of DNA strands and increase the disruption of the orientation profile in the thin LC layer and further induce a homeotropic-to-tiled transition of the LC molecules, resulting in an obvious change of the optical appearance of LC biosensors from a dark background to a birefringent texture. The combination of AuNP-mediated signal amplification and LC-based imaging contributed a high selectivity and offered a significantly lower detection limit (0.1 pM). In a word, this proposed gold nanoparticle based signal enhancement method opens up a new avenue for highly sensitive LC biosensing detection of DNA hybridization, and is of great potential to provide a highly sensitive sensing platform for the study of some specific biomolecular interactions *via* various types of binding events.

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