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COMMUNICATION

A Novel, Label-Free Liquid Crystal Biosensor for Parkinson's Disease Related Alpha-Synuclein

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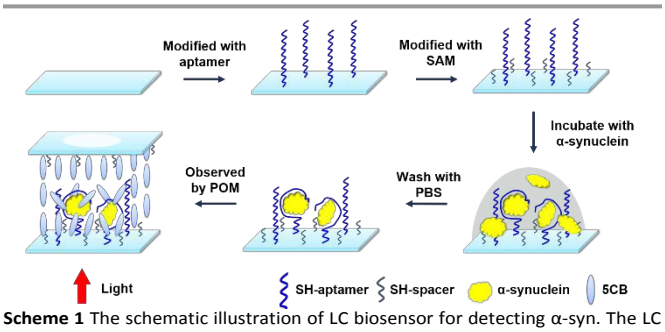
Liquid crystal biosensor based on DNA aptamer for sensitive detection of Parkinson's Disease (PD) related alpha-synuclein was developed. This LC biosensor constructs a simple and label free method, which not only benefits for early PD diagnosis, but also provides a general platform for detection based on DNA aptamer.

With PD being a universal disorder affecting 1-2% of those older than 65 years, early diagnosis of the disease is critical in its prevention and treatment.[1] Extensive research have found that the protein alpha-synuclein (α-syn) plays an important role in the pathogenesis of PD and there is a correlation between Parkinson's disease and the content of α-syn in cerebrospinal fluid as well as blood, making it a potential biomarker for early diagnosis of PD.[2-5] Currently, there are several methods used to detect and visualize the presence of α-syn, such as enzyme-linked immunosorbent assay (ELISA), western blotting (WB) and thioflavin-T (ThT) fluorescence, electrochemical method, etc.[6-10] However, these techniques are usually tedious, costly and require highly skilled personnel to carry out. Therefore, it is desiring but lacking an effective approach to detect α-syn in a simple, label-free and economic way. Herein, we proposed to construct a DNA aptamer based liquid crystal (LC) biosensor to detect α-syn.

Exploitation of LCs for sensing has two advantages: (i) the presence of proteins can cause LC orientational changes which are further transduced into optical signal;[11-15] (ii) LCs can magnify the local interaction from a few molecules at a size of nano meters to a size detected by microscope or even recognized by naked eyes.[16-19] This guarantees that LCs can be used for development of biological sensors.[20-25] In order to enhance the specificity of the interaction between LCs and target α-syn, DNA aptamer was immobilized on solid substrate. Aptamers are single-stranded DNA or RNA sequences that have a unique configuration that specifically recognizes and binds to multiple targets.[26, 27] In 2018, Zheng et al reported a DNA aptamer named as F5R1 that can specifically bind to α-syn with dissociation constant (K_d) of 2.4 nM.[28] Therefore, we anchored this DNA aptamer onto gold coated glass through thiol-gold linkage,[29] see Scheme 1. The background was backfilled with a mixture of $CH_3(CH_2)_9SH$ ($C_{10}SH$) and $CH_3(CH_2)_{15}SH$ ($C_{16}SH$), which has been reported to form a self-assembled monolayer (SAM) and induce homeotropic/perpendicular alignment of LCs.[30] We hypothesize that (i) if there is no α-syn, LCs will keep homeotropic alignment between mixed SAM modified glass, and appear to be dark under the polarized microscope; (ii) if there is α-syn, which will be captured by DNA aptamer and further disrupt the homeotropic alignment of LCs. Optically, LCs will appear bright (planar alignment) under polarized microscope.



Scheme 1 The schematic illustration of LC biosensor for detecting α-syn. The LC cell is composed of two surfaces. The top surface comprised of mixed SAM ($C_{10}SH$+$C_{16}SH$). The bottom surface modified with DNA aptamer which captures α-syn. Under polarized microscope, LCs appear bright in the presence of α-syn, otherwise, keep dark.

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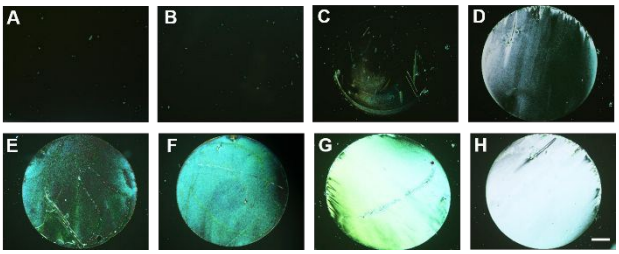

Fig. 1 Optical images (crossed polars) of LC cell in which the bottom glass slide modified with a DNA aptamer solution at a concentration of (A) 0 nM, (B) 45 nM, (C) 50 nM, (D) 100 nM, (E) 200 nM, (F) 300 nM, (G) 400 nM, (H) 500 nM. The circular spot shown in the optical image was caused by the presence of DNA. The scale bar is 250 μm.

First, in order to determine the optimal condition of DNA aptamer anchored on the surface, a series of experiments were carried out. The reported DNA aptamer sequence, F5R1, was used. Considering the immobilization of DNA onto the solid surface may hinder the flexibility of DNA chain, a -$(CH_2)_6$-TTT linker was added at the 5' end of aptamer, allowing DNA strands freely fold upon interaction with α-syn. The detailed sequence information was illustrated in the materials section of ESI†. In a typical experiment, one drop of 2 μL DNA aptamer PBS buffer (pH 7.4) solution with a stated concentration was put onto the gold-coated glass to form a circular spot. After 2 hrs incubation, aptamer solution was rinsed and dried, resulting in DNA aptamer modified gold substrate with a shape of a circular spot at a size of about 1.7 mm. The background was subsequently modified with a mixed SAM ($C_{10}SH+C_{16}SH$). The DNA aptamer modified glass was paired with a glass modified with mixed SAM. The two substrates were separated apart by a 20 μm thick spacer and secured by binder clips. Then, 5 μL LC of 4-cyano-4'-pentylbiphenyl (5CB) in isotropic phase was drawn into the cell via capillary force and slowly cooled to nematic phase. As shown in Fig. 1A, LC cell shows a uniformly dark optical image when there is no DNA aptamer modified onto the glass. This result is consistent with previous reports that mixed SAM ($C_{10}SH+C_{16}SH$) induced homeotropic alignment of LCs.[30] When DNA aptamer was introduced to the surface, Fig. 1B shows that LC cell can still keep dark optical signal at the concertation of 45 nM (refers to the concentration of DNA aptamer solution used for modification), while it appeared bright as the concentration of aptamer solution further increased to 50 nM. This suggested that excessive amount of aptamers immobilized on the glass disrupted homeotropic orientation of LCs.[31] Inspection of Fig. 1C-H revealed that the higher the concentration of DNA aptamer solution, the brighter of LC cell, as more DNA was immobilized on the surface and destroyed the LC alignment. It is acknowledged that a desirable LC based sensor can appear dark (without the analytes) and bright (with the analytes), respectively.[32] Therefore, it is ideal if the DNA aptamer modified LC cell can stay dark under the polarized microscope, suggesting the concentration of DNA aptamer solution should be fixed below 45 nM. On the other hand, in order to capture protein efficiently, DNA aptamer anchored onto the surface should be as much as possible. Considering both factors, the optimal concentration of aptamer solution was determined to be 45 nM, which was used in the following experiments.

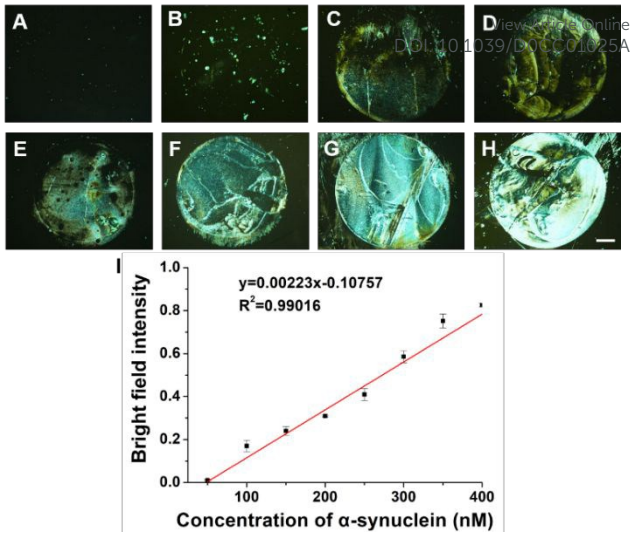

Fig. 2 Optical images (crossed polars) of LC cell in which the bottom DNA aptamer modified glass interacted with α-syn solution at a concentration of (A) 25 nM, (B) 50 nM, (C) 100 nM, (D) 150 nM, (E) 200 nM, (F) 250 nM, (G) 300 nM, (H) 400 nM. (I) Relationship between average bright field intensity and concentrations of α-syn. The scale bar is 250 μm.

Next, we investigated whether the DNA aptamer modified LC cell can detect α-syn. In a typical experiment, 90 μL of α-syn solutions with various concentrations were added onto DNA aptamer modified glass and incubated for 2 hrs. The surface was then rinsed and dried. LC cell was constructed similar to that described previously. Fig. 2 shows visibly clear rim of the spotted circle indicated that α-syn only binds to the area where aptamers immobilized. This phenomenon further verifies that the bright optical signal was due to specific interaction of α-syn with the aptamer instead of non-specific adsorption onto the glass. When the concentration of α-syn fell below 50 nM (Fig. 2A), the amount of α-syn captured by aptamers was too low to change the orientation of LCs, thus the optical signal remained uniformly dark. With a 50 nM concentration of α-syn solution, bright spots started appearing inside the range where aptamers immobilized (Fig. 2B). This indicated that the presence of α-syn and disrupted the homeotropic orientation of LCs at some regions, but due to its relatively low amount, it is insufficient to turn the optical image significantly bright. With the further increase of α-syn solution concentration from 50 nM to 400 nM, the LC sensor gave an increasingly bright optical output (Fig. 2C-H) as there were much more α-syn captured by aptamer. When the concentration of α-syn was over 500 nM, the optical signal was saturated (Fig. S1, ESI†). Based on the above results, it could be concluded that the limit of detection of this biosensor for α-syn was 50 nM.

In order to investigate the quantitative performance of the LC biosensor, the bright field intensity of polarizing optical image was calculated using an established method (the matlab code is shown in ESI†). As depicted in Fig. 2I, the averaged bright field intensity of four samples has a linear relationship with the concentration of α-syn in the range of 50 - 400 nM with a good correlation coefficient over 0.99, suggesting LC biosensor can be applied for quantification of α-syn. Though the detection sensitivity is not superior to existing antibody-based ELISA, the

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LC based biosensor does not require secondary antibodies/enzymatic amplification, showing its advantages of developing a simple and economic biosensor. It is also noted that the optical signal (Fig. 1-2) is dose-dependent and saturable, suggesting the brightness observed is caused by the interaction of target molecule and LCs instead of artefacts.

To further confirm the above-described orientations of LC due to the specific binding of DNA aptamer with α-syn and not, for example, due to non-specific binding of DNA with α-syn, a random DNA sequence was chosen to observe whether it can bind with α-syn and change the orientation of LCs. In an identical experimental procedure, except the DNA aptamer sequence was replaced with 45 nM of random DNA sequence. The result in Fig. S2A (ESI †) showed an almost dark optical image which indicated that the non-specific interaction between random DNA sequence and α-syn was little, as there was no enough α-syn captured by random DNA sequence to induce the bright image of LCs. While under the same condition, the LC cell modified with aptamer gives a much brighter optical image (Fig. S2B, ESI†). These results further confirmed that the aptamer used in this study was highly specific to bind with α-syn rather than through non-specific interaction.

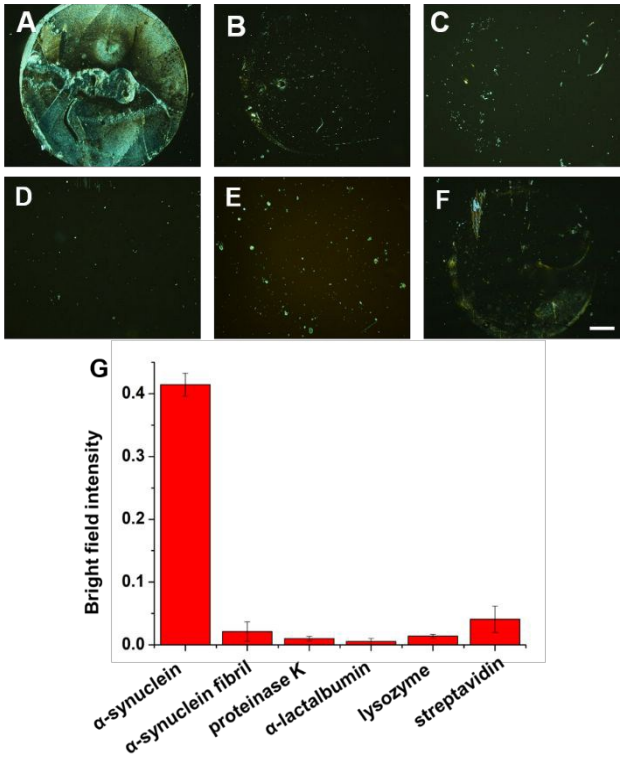

Fig. 3 Optical images (crossed polars) of LC cell in which the bottom DNA aptamer modified glass interacted with 250 nM of different proteins (A) α-synuclein, (B) α-synuclein fibril, (C) proteinase K, (D) α-lactalbumin, (E) lysozyme, (F) streptavidin. (G) Average bright field intensity of 250 nM of α-synuclein and other control proteins. The scale bar is 250 μm.

Finally, we investigate the specificity of our LC sensor, four proteins, such as proteinase K, α-lactalbumin, lysozyme and streptavidin, are chosen as control proteins. We also investigated the interference of α-syn fibrils on the specific detection of α-syn. It was observed that α-syn fibrils with a size of tens nanometers wide and micrometers long[33] (Fig. S3, ESI†), are significantly larger than α-syn monomer which is around several nanometers. If there is any α-syn fibril captured on the surface, the disruption of LCs would be much more impressive comparing to α-syn monomer. From the results shown in Fig. 3, at the concentration of 250 nM, it is obvious that a significant bright optical image can only be generated by α-syn. In contrast, an almost dark optical output was generated for α-syn fibrils and other four proteins. However, weak bright areas were showed in Fig. 3F. As for the control protein streptavidin, in addition to electrostatic effect, the large molecular size of streptavidin (60 kDa), nearly 4 times larger than a α-syn, cannot be neglected. We suspect that due to the much larger size, the streptavidin absorbed on the DNA modified glass could not be washed off easily. Even so, the optical signals output was significantly weaker compared to that of α-syn as showed in the average bright field intensity (Fig. 3G). The experiments suggested that mixed SAM does not introduce severe non-specific adsorption when the protein concentration is relatively low. We also aware that if α-syn increased to 500 nM, α-syn can still adhere to the surface (Fig. S4, ESI†). Therefore, it is stated in Fig. 2 that the possible detection range is up to 400 nM. The non-specific adsorption problem should be addressable by exploitation of non-ionic surfactants and oligo(ethylene glycol).[34, 35]

It is worth noting that the α-syn fibril did not show a bright signal, the reasons are as follows: the DNA aptamer used in this work is specifically for α-syn monomer, not for α-syn fibril, thus the interaction between aptamer and α-syn fibril was not as strong as α-syn monomer. Since the α-syn fibrils were not captured, they were easily washed off at the rinsing step. Based on the above results, it concluded that the LC biosensor has good specificity for α-syn.

In summary, we demonstrated a proof-of-concept that liquid crystals can be utilized for construction of a simple and label-free method for α-syn detection. DNA aptamer specifically binding with α-syn was immobilized onto the surface, as such, the subsequently captured α-syn disrupted the alignment of LCs and resulted in a bright optical image. This LC biosensor showed a lower detection limit of α-syn at a concentration of 50 nM and can quantify α-syn in the range of 50 - 400 nM. Based on this developed general platform, it should be applicable for detection of pathological α-syn in oligomeric forms[36] by using DNA aptamer specifically binding with α-syn oligomers.[37] It is worth exploring LC biosensor to detect α-syn in more complex fluid, such as plasma and cerebrospinal fluid from PD patients. In addition, this work opens the possibility for the detection of a variety of target molecules combined with the development of DNA aptamers.[38, 39]

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Conflicts of interest

There are no conflicts to declare.

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