Electronic Supplementary Material (ESI) for ChemComm.

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

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Experimental Section

Materials

4-Cyano-4'-pentylbiphenyl (5CB) was obtained from Jiangsu Hecheng Display Technology Co., Ltd (Nanjing, China). Lysozyme from chicken egg white, α-lactalbumin from bovine milk, proteinase K from tritirachium, streptavidin form streptomyces avidinii, thrombin from bovine plasma, bovine serum albumin (BSA), phenylmethylsulfonyl fluoride (PMSF), isopropyl-β-D-thiogalactoside (IPTG), coomassie brilliant blue R, 1-hexadecanethiol ($CH_3(CH_2)_{15}SH$), 1-decanethiol ($CH_3(CH_2)_9SH$) and sodium chloride were purchased from Sigma-Aldrich (USA). Plasmaids was bought from Shanghai Heyuan Company (Shanghai, China). Protease inhibitor cocktail (PI) and lysozyme from hen egg white used in purification of recombinant α-synuclein(α-syn) was bought from Roche (Switzerland). Glutathione Sepharose 4B was purchased from GE Heathcare. BL21 (DE3) Chemically Competent Cell (E. coli) was obtained from TransGen Biotech (Beijing, China). Saturated solution of uranyl acetate in water was purchased from Beijing Zhongjingkeyi Technology Co., Ltd. (Beijing, China). The buffer solution used in this paper was PBS (pH 7.4). Other salts and solvents were purchased from Tianjing Fuchen Chemical Reagents Factory (Tianjing, China), which were all analytical grade. DNA aptamers were synthesised by Beijing Qingke Biological Technology Co., Ltd. (Beijing, China). Glass microscope slides (Fisher's Finest Premium grade) were obtained from Fisher Scientific (Pittsburgh, PA). Water used in all experiments was Milli-Q water (18.2 MΩ·cm). Sequence of thiolated aptamer used was: 5' -SH-$(CH_2)_6$ TTTATCGAGTGTGTACGGGGTCCGGTAGGGTGGCGAGGTCTTCCTGTCGTAGCAGGATCCA-3'.
Sequence of thiolated random DNA sequence used was: 5' -SH-$(CH_2)_6$ TTTTCCGAGGCTGGTGGTCTTTACGCGGTACATAAATTCCTACACATCTACGAACCGATAA-3'.

Recombinant Alpha-Synuclein (α-Syn) Expression and Purification

The expression and purification of recombinant α-syn was prepared according to published procedures.[1] The pGEX-4T-2 vector containing cDNA encoded wild-type full-length human α-syn. Plasmids were transformed into BL21 (DE3) Chemically Competent Cell. Bacterial growth was monitored to log-phase, IPTG added for 2 hrs at room temperature rotator, paste collected into lysis buffer (0.1 M PBS) consisting of 300 mM NaCl, 50 mM NaH_2PO_4, 100 × 1 mg/mL lysozyme, 0.2 mM PMSF and 50 × PI. Homogenates were sonicated and tubes placed on ice for 30 minutes. After centrifugation for 30 minutes at 15,000 g, recombinant α-syn was purified by Glutathione Sepharose 4B bead for 16 hrs at 4 °C rotator. Thrombin (25 U) cut beads for 2 hrs at room temperature. Supernatant were collected and concentrated to 2 mg/mL. Protein concentrations were determined by BCA assay and coomassie blue staining using BSA as the protein standard.

Preparation of α-Syn Fibril

α-Syn fibril was prepared by incubating 2 mg/mL α-syn at 37 °C with constant shaking at 1000 rpm (Thermomixer C, Eppendorf) for 7 days. The transmission electron microscopy characterization of α-syn fibril in showed in Fig. S2.

Preparation of Gold-Coated Glass

The glass slides were first cleaned in piranha solution (70% (v/v) H_2SO_4 and 30% (v/v) H_2O_2) for 1 hr at 80 °C. Then the glass slides were cooled to room temperature, rinsed thoroughly with water and dried in an oven at 100 °C for 1 hr. The piranha-cleaned glass slides were evaporated with 10 nm chromium and 40 nm gold (Tsinghua Foxconn Nanotechnology Research Center, Tsinghua, Beijing).

Preparation of Mixed Self-Assembled Monolayer (SAM) Modified Gold Coated Glass

The glass slides were functionalized with SAM using published procedures.[2] Fresh gold-coated glass was first immersed into mixed SAM solution (0.2 mM $CH_3(CH_2)_{15}SH$ and 0.8 mM $CH_3(CH_2)_9SH$ in ethanol) and incubated at 25 °C for 1 hr, after which, the modified glass was rinsed with excess ethanol, followed by PBS buffer. At last, the glass was dried by N_2. The obtained mixed SAM formed on gold surface can induce homeotropic alignment of LCs.

Preparation of DNA Aptamer Modified Gold Coated Glass

One drop of 2 μL DNA aptamer PBS buffer solution with a stated concentration was put onto the gold-coated glass to form a circular spot, followed by incubation at 25 °C for 2 hrs in a water-saturated environment. After incubation, aptamer solution was removed by using a pipette and the glass was rinsed with excess PBS buffer, then dried by N_2. This resulted 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. Then the glass was rinsed with excess ethanol and PBS buffer, then dried by N_2.

Detection of α-Syn

First, 90 μL of α-syn solution with a stated concentration was dropped onto the DNA aptamer modified gold coated glass and incubated for 2 hrs at room temperature in a water saturated environment, allowing aptamers bind with α-syn. After incubation, rinse the glass with PBS buffer to wash off unbound α-syn. Dry the glass with a stream of N_2. Other control proteins were modified in the same way. Second, mixed SAM modified glass was used as the top cover glass of the LC cell, while the aptamer modified glass binding w/wo proteins was used as the bottom glass of LC cell. Pair the top and bottom glass with the modified side facing each other, separate the two glass pieces with a thin layer of polyester mylar of thickness about 20 μm. Secure the paired LC cell with binder clips.[2] 5 μL of 5CB at a temperature around 40 °C

corresponding to its isotropic phase was added to the cell and drawn into paired glass pieces via capillary action. The sample was slowly cooled to room temperature.

Optical Examination of LC Images

A Nikon Eclipse Ti microscope with a digital camera (Nikon DS-U3) in transmission mode was used to observe the optical images of LC cell. The cell was placed on a rotating stage between two polarizers. All images were captured at a resolution of 1600 × 1200 pixel, a gain of 1.00 ×, and a shutter speed of 1/30 s.

Transmission Electron Microscopy (TEM) Characterization of α-syn Fibril

TEM measurements were performed with a JEM-2100 transmission electron microscope. Diluted the fibril sample (2 mg/mL) 50 times with ddH_2O, then dropped 10 μL onto a 200-mesh copper grid. After the sample was dried, put the sample into the 1% (w/v) uranyl acetate for 5 min, then wash it with ddH_2O for 5 min, repeated the washing step for twice. After drying, the sample was observed under electron microscope.

References

1. N. K. Polinski, L. A. Volpicelli-Daley, C. E. Sortwell, K. C. Luk, N. Cremades, L. M. Gottler, J. Froula, M. F. Duffy, V. M. Y. Lee, T. N. Martinez and K. D. Dave, *J. Parkinsons Dis.*, 2018, **8**, 303-322.
2. V. K. Gupta and N. L. Abbott, *Langmuir*, 1996, **12**, 2587-2593.

Investigation of the Saturation of the Optical Signal

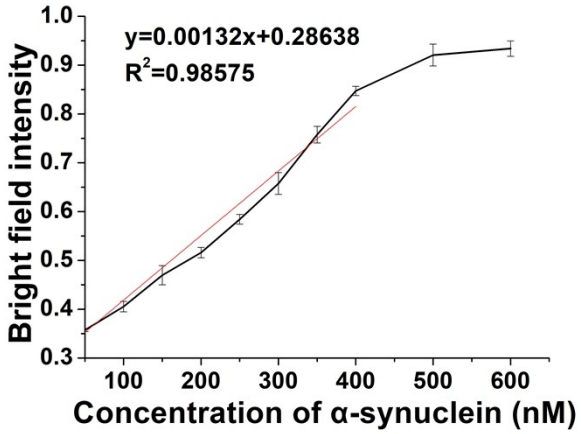

Fig. S1 Relationship between average bright field intensity of the circular spot in the optical image and the concentrations of α-syn.

In order to investigate when the optical signal begins to saturate, we need to preclude the influence of the optical signal of non specific adsorption of α-syn to the SAM over 500 nM. Thus, we calculated the average bright field intensity of the circular spot, not the whole image. The reference value of bright field intensity calculation was 0.4 used here. The results were showed in Fig. S1, when the concentration of α-syn was over 500 nM, the optical signal was saturated. The value of R^2 was slightly lower than that of in the Fig. 2 as the circular drop was not a perfect circle and the size may be slightly different after cutting the image.

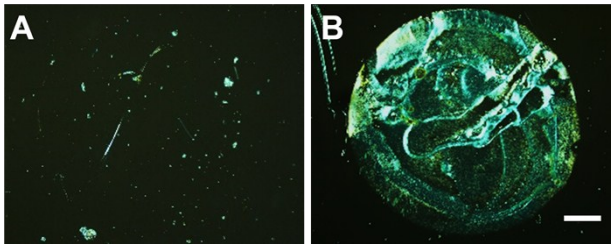

Fig. S2 Optical images (crossed polars) of LC cell modified with 45 nM of (A) random DNA or (B) DNA aptamer after exposed to 300 nM of α-syn. The scale bar is 250 μm.

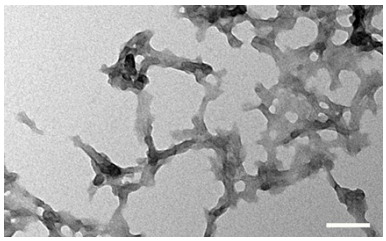

Fig. S3 The transmission electron microscopy characterization of α-syn fibril. The scale bar is 100 nm.

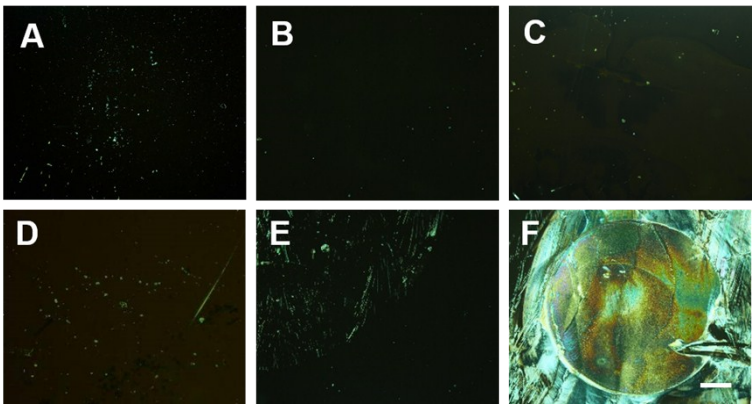

Fig. S4 Optical images (crossed polars) of LC cell in which the bottom glass slide modified with mixed SAM and incubated with (A) α-syn, (B) proteinase K, (C) α-lactalbumin, (D) lysozyme, (E) streptavidin at a concentration of 300 nM. (F) The bottom DNA aptamer modified glass interacted with α-syn solution at a concentration of 500 nM. The bright spots shown in the optical image (A-E) and the bright area outside the circle in (F) was caused by the non-specific adsorption of protein to the SAM. The scale bar is 250 μm.

Matlab Code Used for Characterization of Bright Field Intensity

```
file_path = 'C: ';% Image folder path
img_path_list = dir(strcat(file_path,'*.jpg'));% Get all JPG images in this folder
img_num = length(img_path_list);% Total number of images
Result_Value = zeros(img_num,1);% Preset blank array to give gray value of image
addpath(file_path);
i = 1;% Cycle the gray level of each image and assign it to the cells in the corresponding array
while i <= img_num
    Orig_Picture = imread (img_path_list(i).name);% Read image
    Gray_Picture = im2bw(Orig_Picture,0.2);% Graying color images
    Light_Pixel_Num = nnz (Gray_Picture);% Count the number of pixels in the non-blank area
    [ m, n] = size(Gray_Picture);% Measure the length and width pixel size of gray image
    Picture_Pixel_Num = m * n;% Number of pixels in grayscale image
    Result_Value(i,1)    = Light_Pixel_Num / Picture_Pixel_Num;% Calculate the proportion of non-blank area in the image
    i= i + 1;
end
```