

## Supporting Information

### Partial Induced Reorientation of 5CB in Liquid Crystal Microarray and the Signal-on Sensing Assay for Detection of Aflatoxin B1

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#### Experimental Section

**Materials.** 4-(6-acryloyloxyhexyloxy)-4'-cyano-1 (AO6CB), 1,6-hexanediol diacrylate (C716), acrylic acid (AA), 2-hydroxy-2-methylpropiophenone (HMPP), 4-cyano-4'-n-pentylbiphenyl (5CB), hydrofluoric acid (HF), and dichloromethane (DCM) were purchased from Sigma Aldrich. Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), ochratoxin A (OTA), ochratoxin B (OTB), fumonisin B1 (FB1), zearalenone (ZAE) were purchased from Adamas. All reagents are analytical purity. The SiO<sub>2</sub> nanoparticle suspensions (280 nm) were obtained by the modified Stöber synthesized method. Ultrapure water (18.25 MΩ•cm) was used throughout the experiment. All the oligonucleotides in this paper were prepared by 10 mM phosphate buffer (PB) solution containing 100 mM NaCl. The oligonucleotides were purchased from Sangon Biotech Co., Ltd and these oligonucleotide sequences are listed in Table S1.

**Preparation of the LCM Biosensor.** The SiO<sub>2</sub> particles were first synthesized by the classical Stöber method as reported<sup>1, 2</sup>. Then these monodisperse SiO<sub>2</sub> particles were deposited on the glass by vertical deposition method in anhydrous ethanol to fabricate the close-packed face-centered cubic PhCs templates. Next, the LCP mixture AO6CB, AA, C716, and HMPP was dissolved in DCM and the solvent was gradually volatilized at room temperature. The mixture was injected into a sandwich structure composed of SiO<sub>2</sub> PhCs template by capillary action and polymerized under ultraviolet light for 15 minutes. The polymer films were torn off from the glass and IOLCP films were obtained by removing the PhCs template in 2 wt% HF solution. Finally, the LCM films were fabricated by infiltrating the 5CB in isotropic phase into the IO-LCP films for 5 minutes and cut into single pieces one square millimeter in size after the films dried sufficiently.

**Detection of LCM Biosensor to AFB1.** The AFB1 solution with different concentrations was prepared using PB solution (100 mM NaCl, pH 7.0). And the 100 μM dsDNA<sub>AFB1</sub> composed of the aptamer of AFB1 (C<sub>AFB1</sub>) and the complementary ssDNA (C<sub>C-AFB1</sub>) is annealed at 95°C for 5 minutes and cool naturally to room temperature. Then, the dsDNA<sub>AFB1</sub> solution is mixed with AFB1 solution with different concentrations respectively and reacted at 4°C for 24 hours. Next, the single pieces

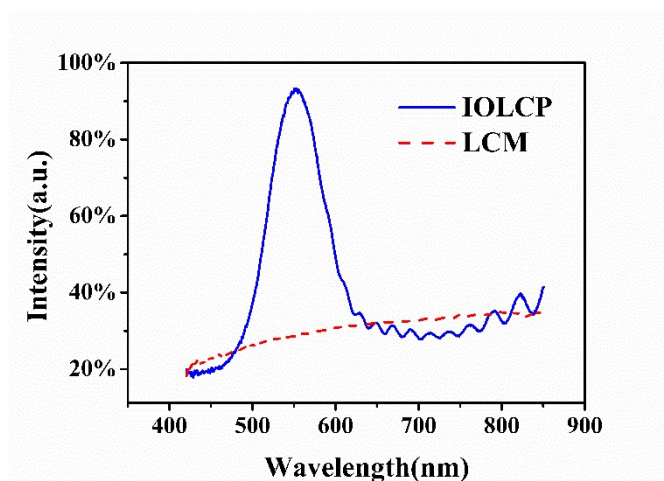
of LCM films were immersed into the above-reacted solution for 2 h at 38 °C in the thermostat. Finally, the reflection peaks of these LCM films were recorded by a fiber-optic spectrometer.

**Detection of AFB1 in Real Samples.** The cornflour was dealt with the methanol and ethanol solution (7:3) firstly and the supernate was filtered with a 0.22 μm filter. Then, these samples 10-fold diluted by 10 mM PB (100 mM NaCl) were prepared, and the AFB1 solution with different concentrations (100 nM, 70 nM, 40 nM, 10 nM) was added into the above solution respectively for 24 h at 4°C. Finally, the LCM films were immersed into these responsive solutions for 2 h at 38°C. And the reflection peaks of these LCM films were recorded by a fiber-optic spectrometer. The beer was filtered with a 0.22 μm filter and further dealt with the same method same as cornflour sample.

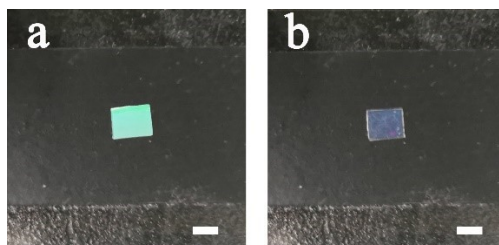
**Apparatus.** Nikon Eclipse polarization microscope (Nikon ECLIPS 50i POL) and MSX20 digital camera; Scanning electron microscope (MIRA4 LMH, TESCAN, Czech Republic); fiber-optic spectrometer (NOVA, Ideaoptics, Shanghai) mounted on a microscope (ECLIPSE 50iPOL, Nikon, Japan); UV light (Scientz 03-II, Scientz, Ningbo); fluorescence inversion microscope system (ZEISS Vert.A1); centrifuging apparatus (Neofuge 23R, Heal Force, Shanghai); circular dichroism spectra (Bio-Logic MOS-500).

**Table S1** The oligonucleotide sequence of C<sub>AFB1</sub>, C14-24, and FAM-C14. All the oligonucleotide strands were purified by HPLC.

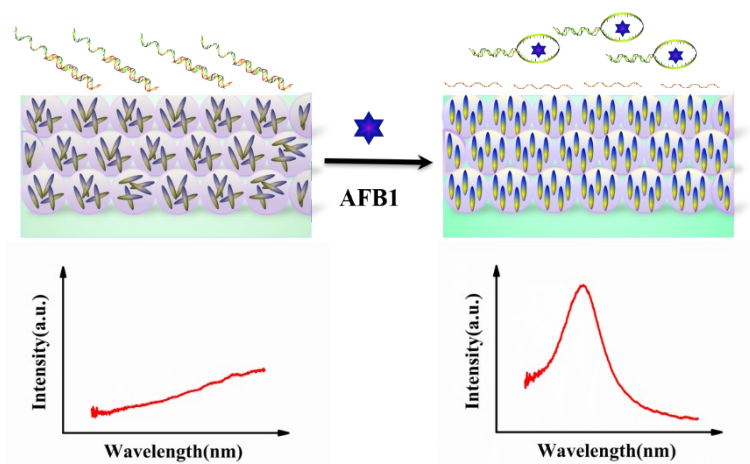
Abbreviation	The oligonucleotide sequence (5'-3')
C <sub>AFB1</sub>	GCTC GTGT TGTC TCTC TGTG TCTC GAC
C14	GAGA CAAC ACG AGC
C16	GAGA GACA ACAC GAGC
C18	CAGA GAGA CAAC ACGA GC
C20	CACA GAGA GACA ACAC GAGC
C22	GACA CAGA GAGA CAAC ACGA GC
C24	GAGA CACA GAGA GACA ACAC GAGC
FAM-C14	6-FAM- GAGA CAAC ACGA GC



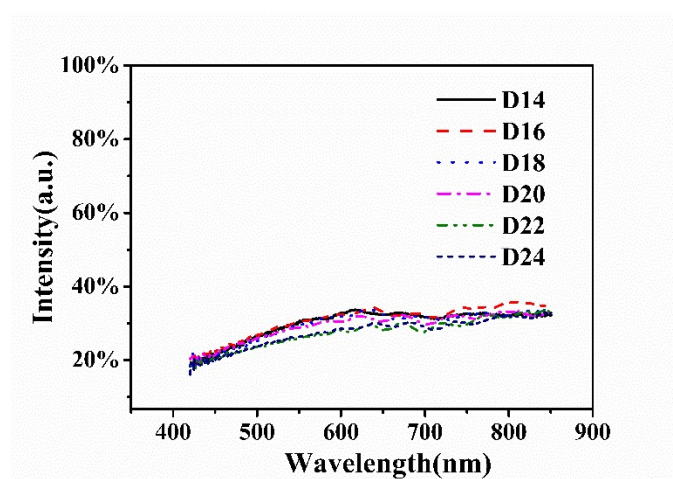
**Figure S1** The reflection peaks of IO-LCP films and LCM films. The obvious reflection peak of IO-LCP films is shown, and reflection peak of LCM not appears.



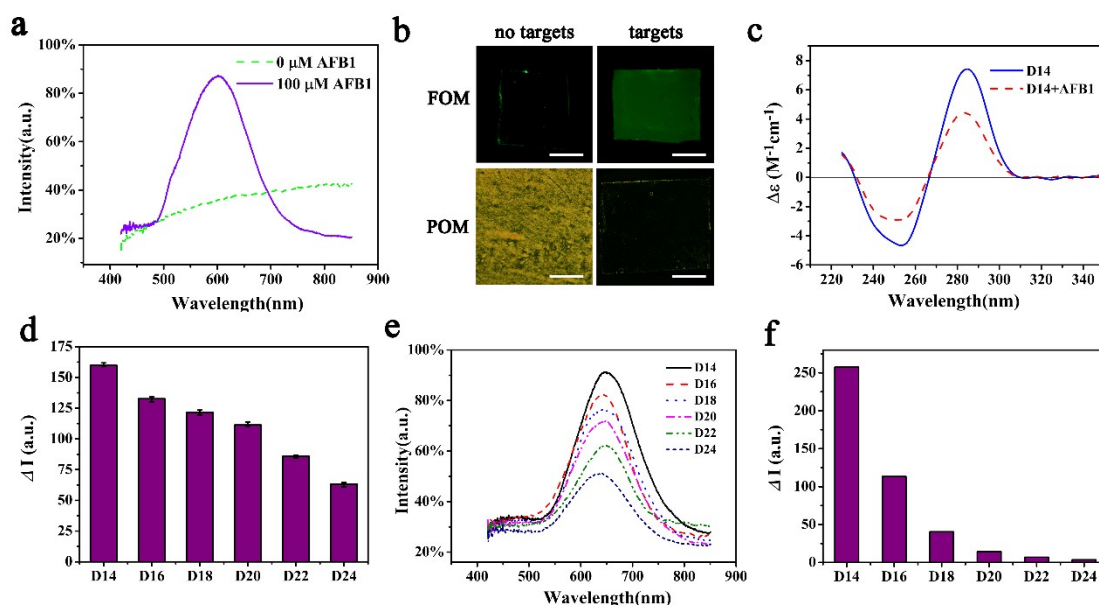
**Figure S2** The real images of IO-LCP films and LCM films. The scale bar is 500  $\mu\text{m}$ .



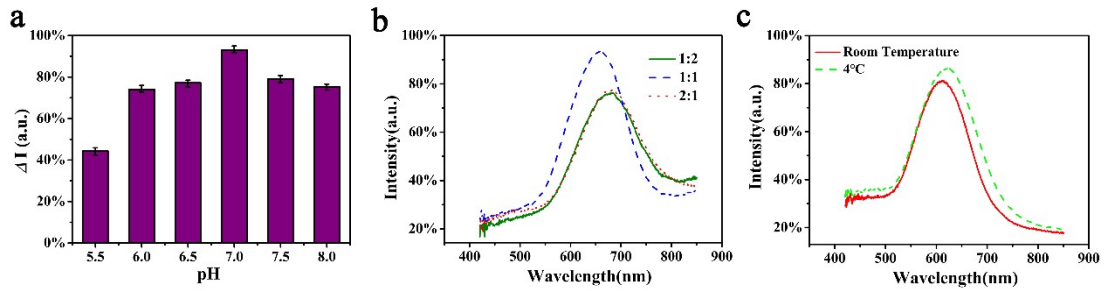
**Figure S3** The schematic diagram of LCM biosensor for AFB1 detection. With the addition of AFB1, the orientation of LC molecules from disordered to ordered arrangement and the intensity of reflection peaks of LCM films increases.



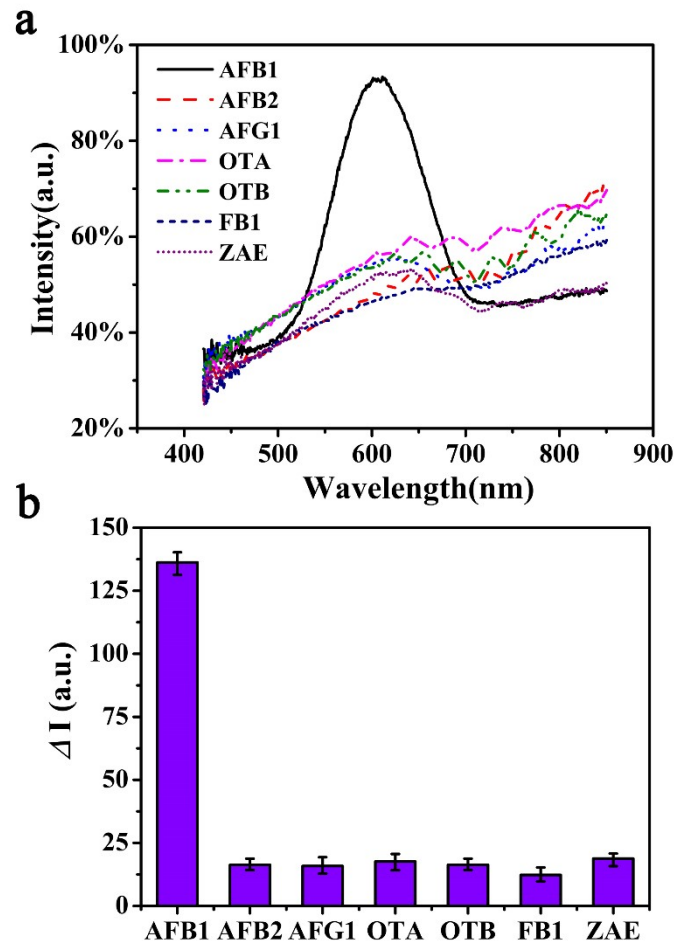
**Figure S4** The influence of dsDNA<sub>AFB1</sub> strands on the response ability of LCM films. The reflection peaks of LCM films when they were immersed into the solution 100  $\mu$ M dsDNA<sub>AFB1</sub> (D14-D24).



**Figure S5** The verification of response mechanism to detect FB1 and the optimization of the number of base groups in complementary strands of aptamer. (a) The reflection peak of LCM films before and after the addition of AFB1. (b) The FOM and POM is shown when the targets are added into the responsive solution before and after. The scale bar is 500  $\mu$ m. (c) The change of CDS of dsDNA<sub>AFB1</sub>(D14) solution with the addition of AFB1. (d) The intensity of reflection peaks of LCM films when the films reacted in dsDNA<sub>AFB1</sub> solution with different base groups number in complementary DNA. (e) The reflection peaks of LCM films when the LCM films were immersed into the solution of 100  $\mu$ M AFB1 and 100  $\mu$ M dsDNA<sub>AFB1</sub> (D14-D24). (f) The change of fluorescent intensity of 100  $\mu$ M dsDNA<sub>AFB1</sub> (D14-D24) dyed by the SYBR Green I after they interacted with the 100  $\mu$ M AFB1.



**Figure S6** The optimization of LCM biosensor. (a) The intensity changes of reflection peaks of LCM films after they interacted with the solution of 100  $\mu$ M AFB1 and 100  $\mu$ M D14 in different pH value from 5.5 to 8.0. (b) The reflection peaks of LCM films when the concentration ratio of  $C_{C\text{-AFB1}}$  and  $C_{\text{AFB1}}$  (1:2, 1:1, 2:1) is used to reacted with 100  $\mu$ M AFB1. (c) The reflection peaks of LCM films in different reacted temperature of 100  $\mu$ M AFB1 and 100  $\mu$ M D14.



**Figure S7** The selectivity of LCM biosensor to AFB1. (a) The reflection of LCM films in different interference substances, including AFB2, AFG1, OTA, OTB, FB1, and ZAE. The concentration of these interference substance is 100  $\mu$ M. (b) The intensity of reflection peaks of LCM films response with AFB1 and interference substances.

Table S2 A overview on recently reported optical/electrochemical determination of AFB1.

Materials used	Methods	LODs	Reference
AuNPs/DNA composites	Fluorescence	0.01 nM	3
Thionine-graphene nanocomposite	Electrochemistry	0.01 ng/mL	4
Gold electrode	Electrochemistry	2 nM	5
Gold nanoparticles	Colorimetry	1.88 nM	6
Gold electrode	Electrochemistry	30 pM/mL	7
Magnetic nanoparticles	Nanozyme Linked Immunosorbent Assay	0.54 fg/mL	8
Liquid crystal Microarray	Optical sensor	300 pM	This work

Table S3 Results of the Determination of AFB1 in Samples and Recovery Test

sample	added (nM)	found (nM)	Recovery, RSD (n=3, %)
corn flour	10	9.7	97
	40	39.2	98
	70	69.3	99
	100	98	98
beer	10	9.7	97
	40	38.8	97
	70	67.2	96
	100	97	97

## Reference

1. Y. J. Wong, L. F. Zhu, W. S. Teo, Y. W. Tan, Y. H. Yang, C. Wang and H. Y. Chen, *J. Am. Chem. Soc.*, 2011, **133**, 11422-11425.
2. R. S. Fernandes, I. M. Raimundo and M. F. Pimentel, *Colloids Surf. A-Physicochem. Eng. Asp.*, 2019, **577**, 1-7.
3. B. Wang, J. Zheng, A. Ding, L. Xu, J. Chen and C. M. Li, *Food Chemistry*, 2019, **294**, 19-26.
4. Y. Li, D. Liu, C. Zhu, M. Wang, Y. Liu and T. You, *Sensors and Actuators B-Chemical*, 2021, **336**.
5. C. Wang, Y. Li and Q. Zhao, *Biosens. Bioelectron.*, 2019, **144**.
6. Y. Yang, Y. Yin, X. Li, S. Wang and Y. Dong, *Sensors and Actuators B-Chemical*, 2020, **319**.
7. C. Zhu, D. Liu, Y. Li, S. Ma, M. Wang and T. You, *Biosens. Bioelectron.*, 2021, **174**.
8. D. Lu, H. Jiang, G. Zhang, Q. Luo, Q. Zhao and X. Shi, *Acs Applied Materials & Interfaces*, 2021, **13**, 25738-25747.