Supporting information for

Effect of organic solvents on the immunosensing assay of small molecules based on optofluidic immunosensing platform

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Materials and chemicals

Bisphenol A(BPA), (3-Aminopropyl)-trimethoxysilane (APTES), Glutaraldehyde (GA), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Methylbenzene, methanol, acetone, acetonitrile, n-hexane, H_2SO_4, H_2O_2, and all other reagents, unless specified, were supplied by Beijing Chemical Agents. All reagents were used as received. Distilled deionized water was used throughout the investigation. 0.01M phosphate buffer solution (PBS, pH=7.4, 137 mM NaCl, 2.7mM KCl, 4.3mM Na_2HPO_4, and 1.4mM KH_2PO_4) was used. BPA-BSA, and AFM_1-OVA were produced by our research group.

Immobilization of hapten-carrier protein conjugates onto biosensor surface

The fabrication of the fiber optic biosensor was prepared according to the previously described method [1]. Briefly, the silica optical fiber with the core diameter of 600 μm (purchased from Chunhui Science&Technology Co., Nanjing, China) had a length of 5 cm, we remove about 2.5 cm of the coating layer, and then put it into the 30% of hydrofluoric acid for about 3h to get a fiber optic sensor with a combination tapered structure based on tube etching.

The hapten-carrier protein conjugates (BPA-OVA or AFM_1-OVA), served as bio-recognition molecules, were immunobilized onto the silanized optical fiber sensor surface using the glutaraldehyde-covalent-coupling strategy (Figure S2) [2]. Briefly, Initially, the sensor was washed by piraha solution (H_2SO_4:H_2O_2=3:1,v/v) 30 min to remove possible contaminants and introduce hydroxyl group on the probe surface, and then washed using ultrapure water three times. After dried, the fiber optic sensor was aminated by immersing in 2% (v/v) APTS in acetone solution for 1 h to coat a reactive silane layer with an aminated-terminal silane. Excess APTS was eliminated with acetone. Then, the sensor was immersed in a 5.0% (v/v) GA solution at 37 ºC for

1 h. After washed with ultrapure water, the sensor was immersed overnight in 1 mL of 0.5 μM BPA-BSA (or AFM_1-OVA) in PBS solution at 4 ºC. Finally, the sensor was placed into a 2 mg/mL BSA solution for 2 h to block the non-specific binding sites. The prepared biosensor was stored at 4 ºC.

Figure S1. The immobilization process of coated-antigen onto the sensor surface through the glutaraldehyde covalent coupling approach.

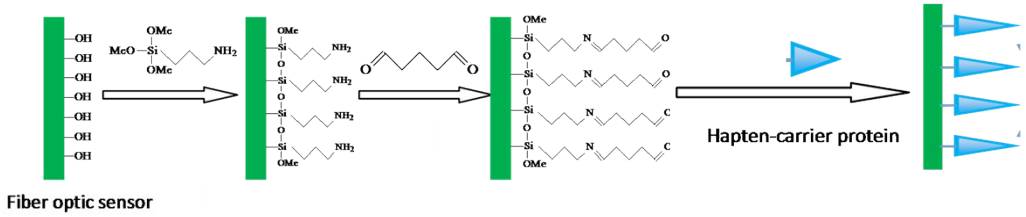


Figure S2. Evanescent wave optofluidic immunosensing platform

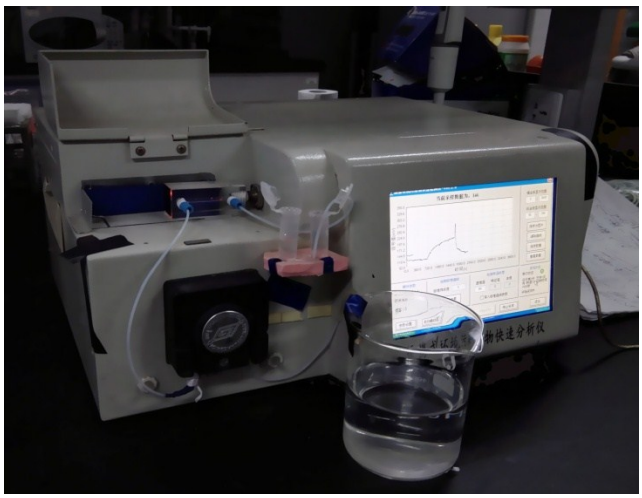


Figure S3. Characterization of optic fiber biosensor modified by the AFM_1-OVA conjugates

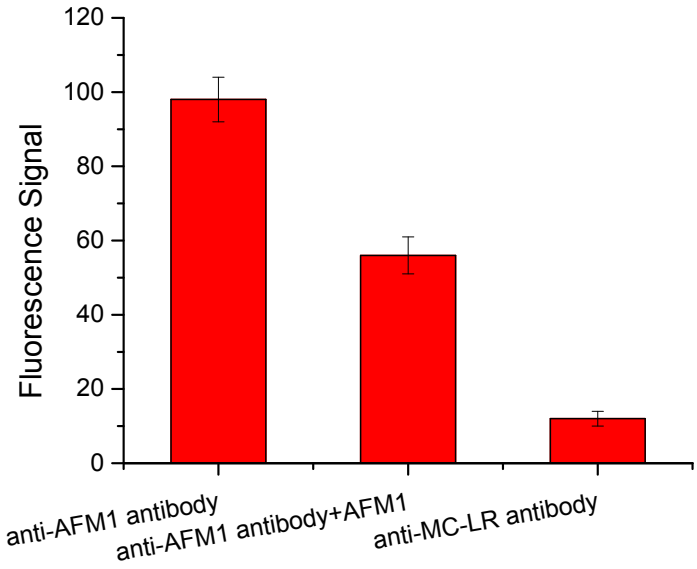


Figure S4. Reusability of immunosensor modified by hapten-carrier protein (AFM_1-OVA) conjugates

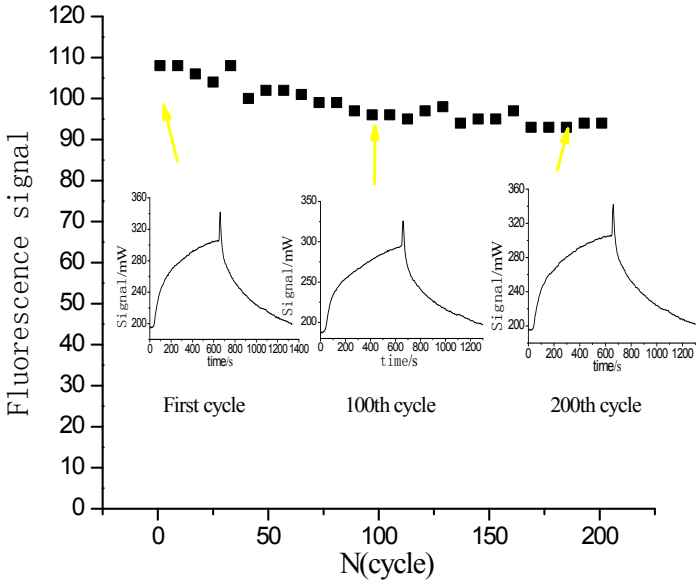


Figure S5 Indirect competitive immunoassay of small molecules

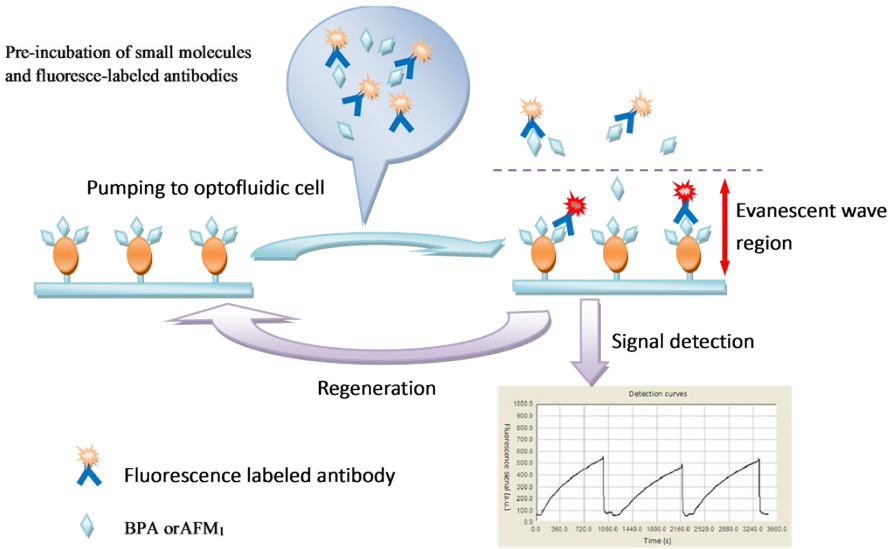
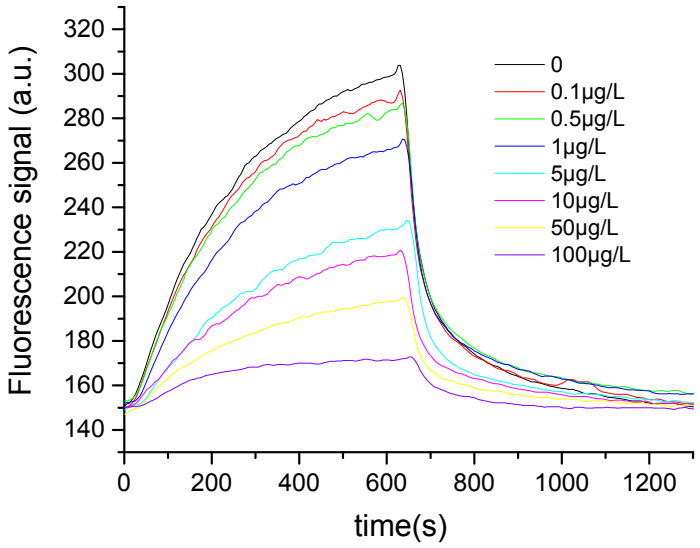
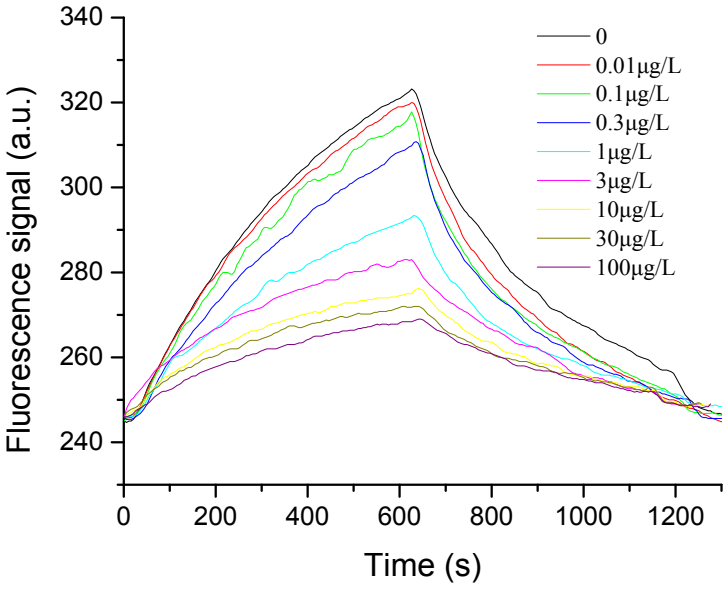


Figure S6. The detected fluorescence signal inversely decreases with increasing of BPA (or AFM_1) concentration. (a) BPA immunoassay; (b) AFM_1 immunoassay.



(a)



(b)

Figure S7 Effect of acetonitrile on the property of Cy5.5. The concentration of acetonitrile ranged from 0% to 40%.

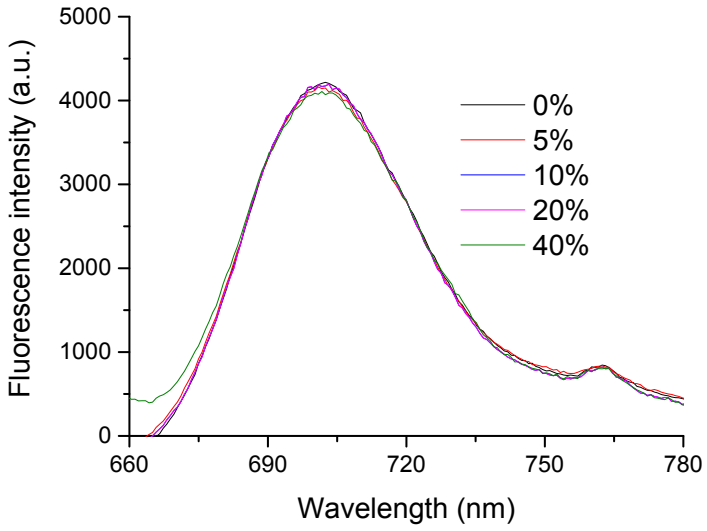


Table S1. The absolute value of the linearly fitted curve slope of BPA immunoassay in the presence of methanol was added into the mixture.

Methonal concentration (v/v, %)	0	1	2	5	10	20	30	40
The absolute value of the linearly fitted curve slope	0.368	0.398	0.379	0.376	0.368	0.267	0.242	0.200

Table S2. The standard deviation (R^2) of linear fitting curve of BPA immunoassay in presence of acetone or acetonitrile

Organic solvent Concentration (v/v, %)	0	1	2	5
Acetone (R^2)	0.992	0.987	0.925	0.608
Acetonitrile (R^2)	0.998	0.978	0.893	