Supporting Information

A pendant droplet-based sensor for detection of acetylcholinesterase and its inhibitors

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Experimental

Materials

Acetylcholinesterase (AChE), trishydroxy-methyl aminomethane (Tris), neostigmine bromide, rivastigmine, galantamine, O,O-dimethyl-O-2,2-dichlorovinyl phosphate (DDVP), lipase (100-150 U/mg), urease (300 U/mg) and catalase (≥200 U/mg) were purchased from Sigma-Aldrich. 4-cyano-4'-pentylbiphenyl (5CB) and dimethoate were provided by J&K Scientific Co., Ltd., China. Myristoylcholine chloride (Myr), cellulase (200 U/mg) and pepsin (>3000 U/mg) were obtained from Shanghai Shifeng Biological Technology Co., Ltd, China. Bovine serum albumin (BSA) was obtained from Shandong Aibo Technology Trade Co., Ltd., China. N,N,N-trimethyl-1-dodecanaminium bromide (DTAB), tetradecyl trimethyl ammonium bromide (TTAB) and cetyl trimethyl ammonium bromide (CTAB) were provided by Sinopharm Chemical Reagent Co., Ltd, China. 10 μL liquid phase microsyringe (flat head, outer diameter: 0.7 mm, inner diameter: 0.15 mm, needle length: 51 mm) was purchased from Shanghai Gaoge Industry and Trade Co., Ltd., China. Ibuprofen, levofloxacin, cephalothin, roxithromycin, amoxicillin trihydrate and Hg^{2+} solution (100 μg/mL) were obtained from Shanghai Macklin Biochemical Co., Ltd., China. Pb^{2+} standard (100 μg/mL) was obtained from Rhawn Technology Co., Ltd., China.

Measurement of the surfactant effect on the pending time of the 5CB droplet

Initially, 6 μL of 5CB was slowly transferred into 500 μL of the surfactant solution in a glass vial using a microsyringe that was secured to the top of the vial by a slot at 25℃. The experimental setup was provided in Figure S1. Then, the pending

time of the surfactant was recorded using a camera. The aqueous solutions of different surfactants were examined to investigate their effect on the pending time of the 5CB droplet. All the aqueous solutions were prepared in Tris-HCl buffer (TBS, 10 mM Tris, pH=7.4). For the optimization of the volume of the 5CB droplet, the 5CB droplets with different volumes were in contact with the aqueous solutions of 0.3 mM Myr, respectively.

Measurement of the interfacial tension

The measurement of the interfacial tension was carried out using a KRÜSS drop shape analyzer – DSA100 (KRÜSS, Germany) with a video camera mounted on the microscope to record the droplet image. 5CB was automatically injected into the aqueous solution by controlling the microsyringe fixed on the instrument using the software. The images of the droplet were recorded at different time after the stabilization of the droplet for a period of time, when the droplet became pear-shaped with the bond numbers between 0.5 and 0.6. The calculation of the interfacial tension is based on the analysis of the experimental drop shape derived from the Yang-Laplace equation.

Detection of AChE and its inhibitors

For the detection of AChE, different aqueous solutions of AChE (0.1-1000 mU/mL) were added into 0.3 mM Myr solutions and incubated at 37 °C for 30 min. Then, the pending time of the 5CB droplet in these solutions was examined. For the detection of AChE inhibitors [13-15], the aqueous solutions of AChE and its inhibitors were individually pre-incubated at 37 °C for 30 min. Afterwards, each

mixture was added into the 0.3 mM Myr solution and incubated at 37 °C for 30 min before examining the pending time of the 5CB droplet.

Specificity tests of the pendant droplet sensor for the detection of AChE inhibitors

In order to verify the specificity of the pendant droplet sensor for the detection of AChE inhibitors, we pre-incubated aqueous solutions of 1 mU/mL AChE and different drugs and small molecules such as ibuprofen, levofloxacin, cephalothin, roxithromycin, amoxicillin trihydrate, serotonin, dopamine, ascorbic acid and acetylcholine at 37 ℃ for 30 min. The concentrations of the drugs and small molecules were 0.01 M. Then, the aqueous solutions were separately mixed with 0.3 mM Myr solutions in a volume ratio of 1:1 (final volume is 500 μL). Afterwards, the pending time of the 5CB droplet was examined individually.

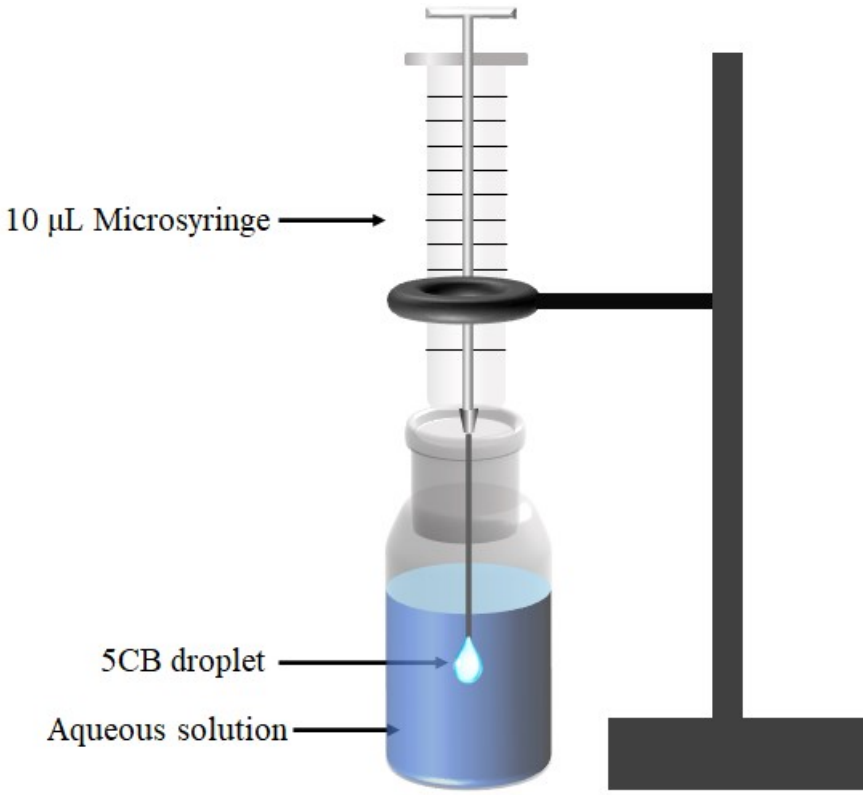


Figure S1. The schematic illustration of the experimental setup.

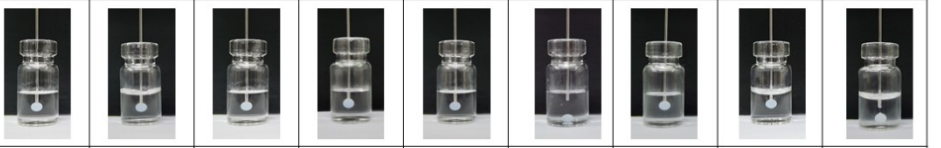
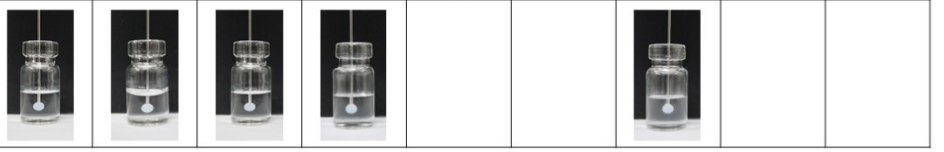
Sample / Time	DTAB			TTAB			CTAB		
	0.1 mM	0.3 mM	0.5 mM	0.1 mM	0.3 mM	0.5 mM	0.1 mM	0.3 mM	0.5 mM
60 s									
600 s									
1200 s									

Figure S2. The responses of the pendant 5CB droplets to different surfactant solutions.

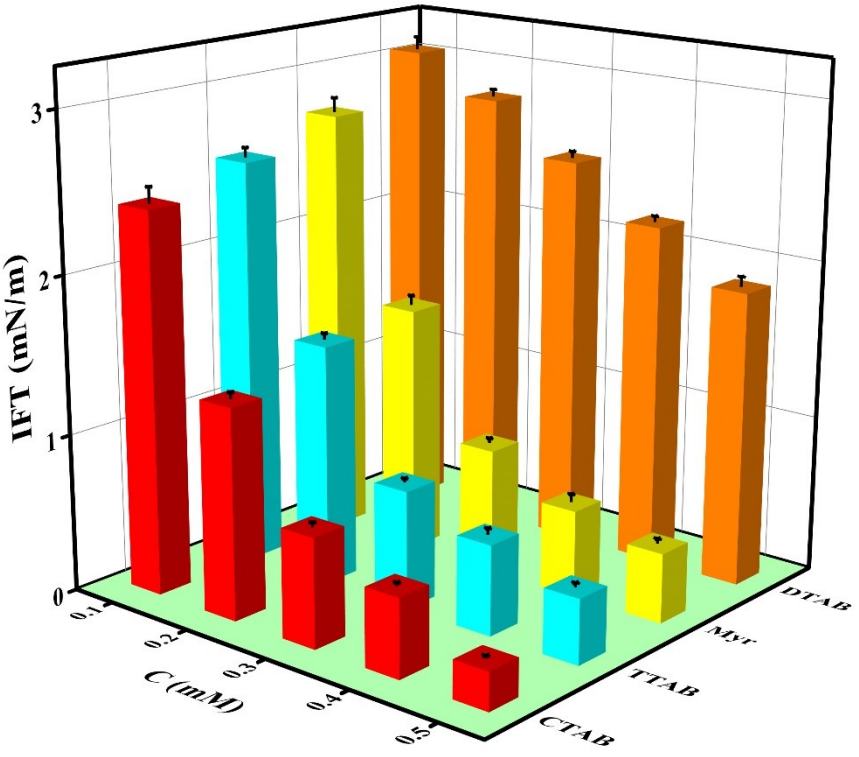


Figure S3. The interfacial tensions of the different surfactant solutions measured by the pendant droplet tensiometry, respectively.

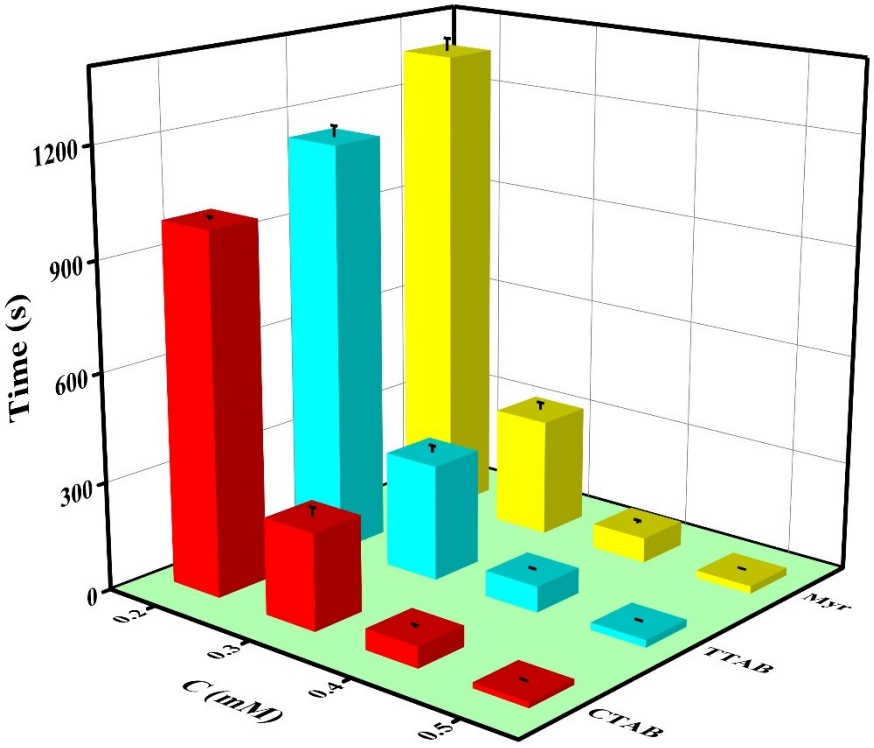


Figure S4. The pending time of the 5CB droplet in the CTAB, TTAB and Myr solutions of different concentrations, respectively.

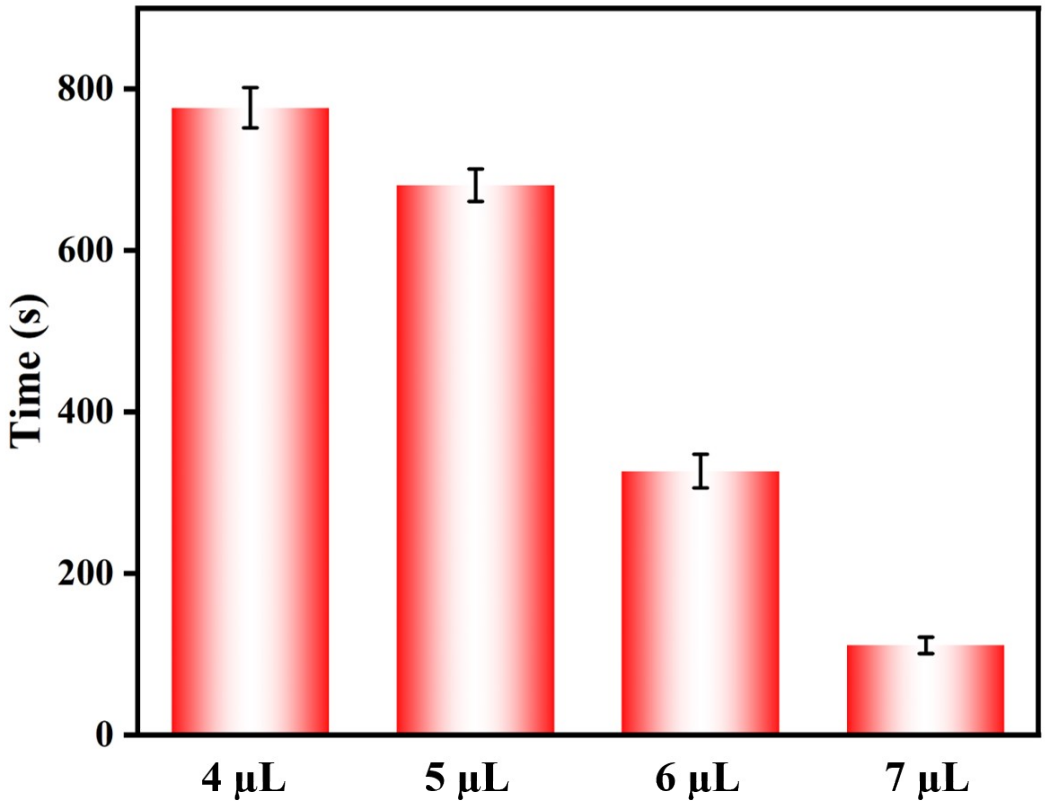


Figure. S5. The pending time of the 5CB droplets with different volumes in 0.3 mM Myr solutions, respectively.

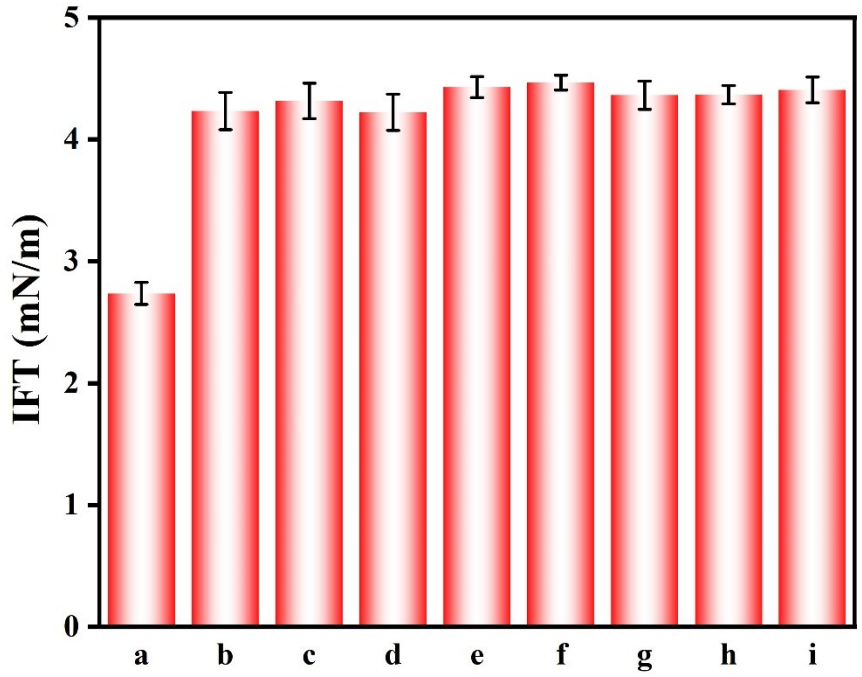


Figure. S6. Interfacial tensions of the pendant droplets in (a) 0.1 mM Myr, (b) TBS, and the aqueous solutions of different inhibitors: (c) Pb^{2+}, (d) Hg^{2+}, (e) DDVP, (f) dimethoate, (g) neostigmine, (h) galantamine, (i) rivastigmine.

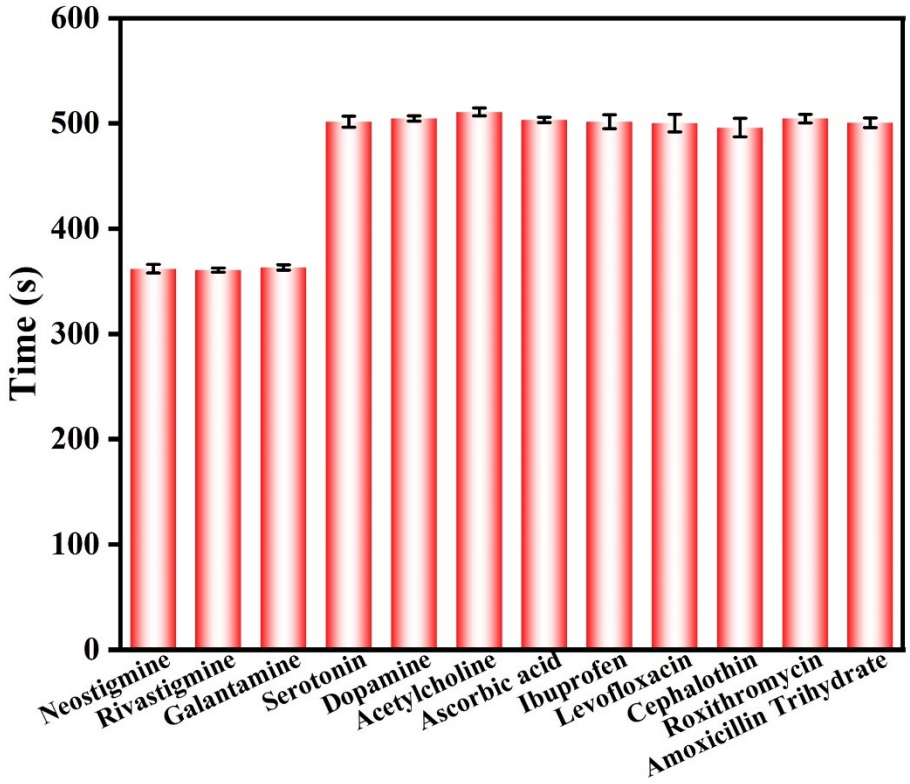


Figure S7. The specificity tests of the pendant droplet sensor in responses to different drugs and other molecules. The data are presented as mean ± SD (n = 3).

Table S1. Comparison on the detection limits of AChE in this work and previous studies.

Detection method	Linear range (mU/mL)	Detection limit (mU/mL)	Ref.
Fluorescent	0-16	1.78	1
Fluorescent	0.02-1	0.15	2
Fluorescent	0.4-25	0.15	3
Fluorescent	15-500	4	4
Luminescent	10-10000	5	5
Luminescent	5-150	2	6
Luminescent	5-100	5	7
Luminescent	1-1000	1	8
Colorimetric	0.01-1	0.12	9
Colorimetric	0.075-25	0.11	10
Colorimetric	0.6-26	1	11
Colorimetric	0.5-10	0.17	12
Pendant droplet	0.1-1000	0.17	In this work

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