Supporting Information

Ochratoxin A Enhanced Detection of Cytochrome c with Aptamers-Based Microcantilever Sensor

Chemicals and Materials

Cyt c and MCH were purchased from Sigma-Aldrich (St. Louis, MO., USA). OTA was bought from Pribolab Pte. Ltd. (Singapore). And tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was obtained from Alfa Aesar (USA). Aptamers (Lau et al. 2010) (5'-(SH)-$(CH_2)_6$--CCG TGT CTG GGG CCG ACC GGC GCA TTG GGT ACG TTG TTG C-3') were synthesized by Sangon (Shanghai, China) and dissolved in immobilization buffer (10 mmol/L PBS containing 187 mmol/L NaCl, 2.7 mmol/L KCl, 8.1 mmol/L Na_2HPO_4, 1.76 mmol/L KH_2PO_4, and 100 μmol/L TCEP (tris(2-carboxyethyl)phosphine), at pH 7.0). The binding buffer [1] for the specific interaction of cyt c and aptamers were prepared by 187 mmol/L NaCl, 2.7 mmol/L KCl, 8.1 mmol/L Na_2HPO_4, 1.76 mmol/L KH_2PO_4 and pH 7.0. Commercial Cantisens sensor platform (Concentris GmbH, Switzerland) equipped with a flowing cell was used to measure the deflection of microcantilevers. The circular dichroism (CD) spectra were collected by a JASCOJ-810 spectropolarimeter[2]. Millipore MilliQ water was used in all the experiments. The binding buffer that flowed through the liquid system was filtered by 0.22 μm syringe filters to avoid blocking the pipe in the liquid cell.

Functionalization of microcantilever sensors

The array contains eight identical microcantilevers that made of mono crystalline silicon with a 20 nm coating of gold on one side and each microcantilever is 500 μm long, 90 μm wide, 1 μm thick (Micromotive GmbH, Mainz, German). The microcantilevers would bend with the change of surface stress induced by probes on the surface with targets in the solution. In this work, the microcantilevers bending towards the gold side is defined as positive and the opposite side as negative. Prior to functionalization procedure, the microcantilever array was cleaned by Piranha solution (98% H_2SO_4:30% H_2O_2, 7:3. Caution! Piranha has strong oxidation and corrosion and must be prepared carefully) for 30 seconds, flowed by 30 minutes of ultraviolet ozone cleaning. Then the gold surface of four microcantilevers (**1**, **3**, **5** and **7**) was modified with aptamers of 1 μmol/L for 3 hours. To eliminate the interference from environment, such as the flow of the liquid, the ion strength in the solution and the unspecific absorbance on the microcantilevers, the other four microcantilevers work as the reference by immersing the whole array in 2 mmol/L MCH dissolved in ethanol. The functionalization schematic was shown in the magnified image in Scheme 1(the details of the microcantilevers of **1** and **2** on behalf of sensing and reference microcantilevers, respectively)

The measurement of deflection of microcantilever array

Followed by the functionalization and backfill procedures, the microcantilever array was mounted on a holder that inserted into the cell. The temperature of cell was

maintained at 25.00±0.01°C by an automatic temperature calibration system equipped with Cantisens. The binding buffer flowed through the liquid system at a flow rate of 0.42 μL/s. The sample was injected into the cell after the baseline was stable. The deflection of the microcantilevers induced by the changing of the surface stress was recorded versus time.

The average deflection curves microcantilevers

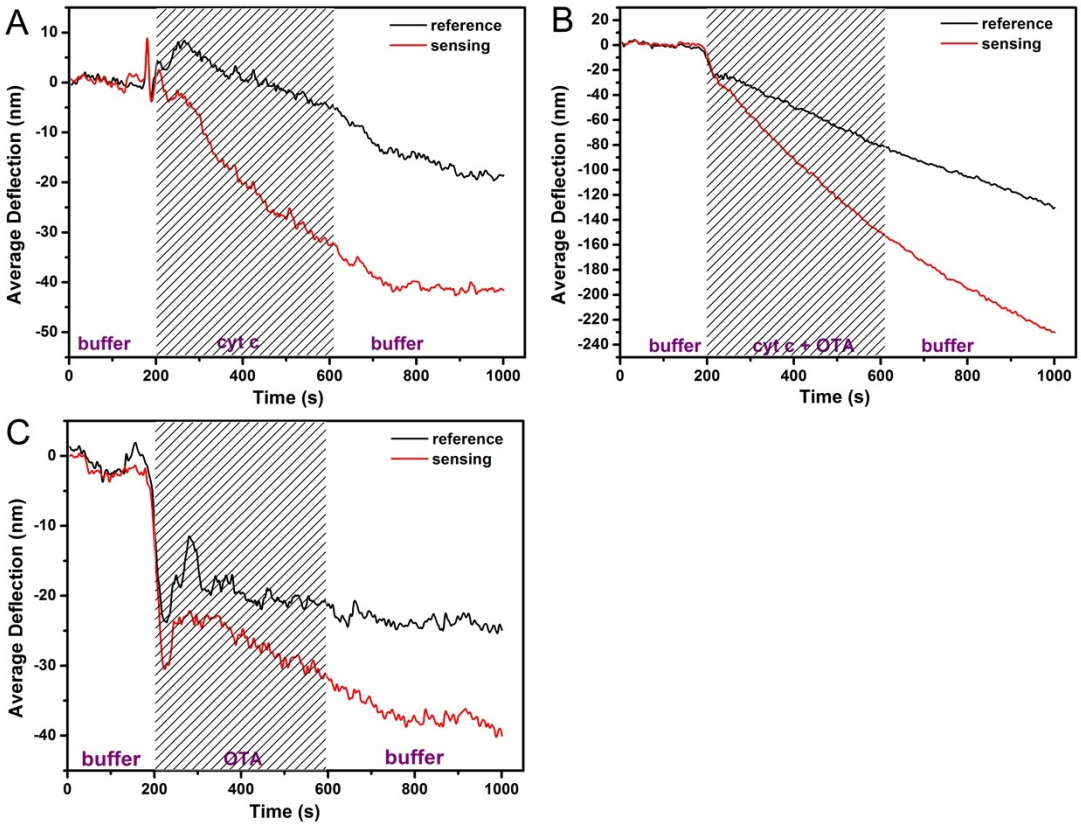

Fig. S1. The averaged deflection curves of sensing (red) and reference (black) microcantilevers induced by the injection of (A) 10μmol/L cytochrome c, (B) the mixture of 10 μmol/L cyt c and 5 μg/mL ochratoxin A, and (C) 5 μg/mL OTA, respectively.

The optimality conditions of detecting cytochrome c by microcantilever sensor

We investigated the optimality conditions of detecting cytochrome c by microcantilever sensor. The effect of aptamer concentration on microcantilever deflection was showed in Fig. S2A. The deflection gradually increased along with the increase of aptamer concentration. When the concentration reached 0.75 μmol/L, the deflection reached a platform, indicating the whole cantilever had been functionalized by aptamers. Thus 1 μmol/L of aptamer concentration was applied in the further experiments.

The effect of incubation time of microcantilevers modified by the aptamers was investigated as showed in Fig. S2B. The deflection increased along with the increase of incubation time. When the incubation time reached 3 h, the current reached a maximum, indicating the most aptamers had been immobilized. So we chose 3 h as the optimal condition in all the experiment.

Fig. S2C illustrated the influence of pH in the reaction buffer on microcantilever deflection for detecting cyt c. The experimental results indicated that pH 7.4 were favorable for this chemical system. Then we finally chose it as the optimal experiment conditions.

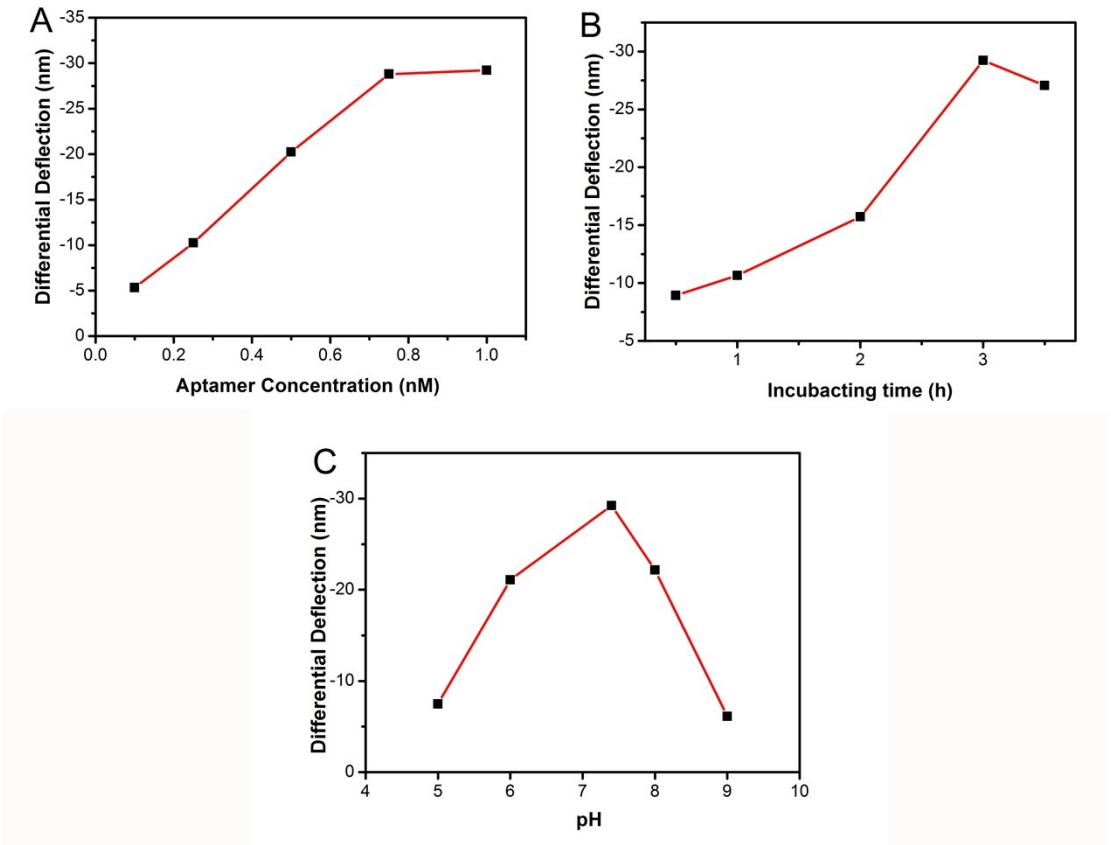

Fig. S2. The optimality conditions of detecting cytochrome c by microcantilever sensor. (A) Effect of the aptamer concentration on microcantilever deflection for detecting cyt c; (B) Effect of the incubation time for the microcantilevers in the aptamers on microcantilever deflection for detecting cyt c; (C) Effect of the reaction buffer pH on microcantilever deflection for detecting cyt c.

References

1. V. Stepanova, D. Shurpik, V. Evtugyn, I. Stoikov, G. Evtugyn, Y. N. Osin and T. Hianik, *Sens. Actuators B Chem.*, 2016, **225**, 57-65.
2. I. P. M. Lau, E. K. S. Ngan, J. F. C. Loo, Y. K. Suen, H. P. Ho and S. K. Kong, *Biochem. Biophys. Res. Commun.*, 2010, **395**, 560-564.